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(54) Title: METHODS FOR PROPHYLAXIS AND TREATMENT OF HER-2/*neu* TUMORS

(57) Abstract: The invention provides for methods for vaccination of dendritic cells, modified by recombinant adenoviral vectors (rAds) expressing truncated HER2/*neu* genes. Infection of dendritic cells with recombinant adenoviral vectors increases the expression of the DC surface maturation markers CD80, CD86, MHC-class I, II; and CD40 and spontaneous breast cancers were delayed or prevented by vaccination with dendritic cells genetically modified by recombinant adenoviruses expressing a non-signaling HER2/*neu* antigen.

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METHODS FOR PROPHYLAXIS AND TREATMENT OF HER-2/*neu* TUMORS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application U.S.S.N. 60/422, 395, filed October 30, 2002, the entire contents of which, are hereby incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH

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BACKGROUND OF THE INVENTION

1. Field of the Invention.

The invention provides a method for treating a subject suffering from cancer or is identified as being susceptible to developing cancer. In particular aspects, a recombinant E1-, E3-deleted non-replicating adenoviral gene transfer vector expressing a truncated HER-2/*neu* oncogene is effective as a vaccine against HER-2/*neu* expressing breast cancer cells and other tumors.

2. Background.

Based on the National Cancer Institute (NCI) incidence and National Center for Health Statistics (NCHS) mortality data, the American Cancer Society estimated that breast cancer would be the most commonly diagnosed cancer among women in 2002 in the United States. It is expected to account for 31 percent (203,500) of all new cancer

cases among women and 39,600 will die from this disease. Unfortunately, only about 50% of the breast cancers are localized at the time of diagnosis. Despite the availability and recommended use of mammography for women age 40 and older as a routine screening method, its effectiveness on reducing overall population mortality from breast cancer is still being investigated.

During the last decade, a number of human malignancies have been identified as correlated with the presence and expression of "oncogenes" in the human genome. More than twenty different oncogenes have now been implicated in tumorigenesis, and are thought to play a direct role in human cancer. Many of these oncogenes apparently evolve through mutagenesis of a normal cellular counterpart, termed a "proto-oncogene", which leads to either an altered expression or activity of the expression product. There is considerable data linking proto-oncogenes to cell growth, including their expression in response to certain proliferation signals and expression during embryonic development. Moreover, a number of the proto-oncogenes are related to either a growth factor or a growth factor receptor.

An oncogene referred to variously as c-erbB-2, HER-2 or *neu* oncogene (referred to herein simply as the *neu* oncogene), is now known to be intimately involved in the pathogenesis of cancers of the human female breast and genital tract. The *neu* oncogene, which encodes a p185 tumor antigen, was first identified in transfection studies in which NIH 3T3 cells were transfected with DNA from chemically induced rat neuroglioblastomas. The p185 protein has an extracellular, transmembrane, and intracellular domain, and therefore has a structure consistent with that of a growth factor receptor. The human *neu* gene was first isolated due to its homology with v-erbB and EGF-r probes (Semba K et al., *PNAS*, USA 82(19):6497-501 (Oct.1985); King, CR et al, *Science* 229(4717):974-6 (Sept. 6,1985)).

The *neu* oncogene is of particular importance to medical science because its presence is correlated with the incidence of cancers of the human breast and female genital tract. Moreover, amplification/overexpression of this gene has been directly

correlated with relapse and survival in human breast cancer (Slamon DJ, et al. *Science* 235(4785):177-82 (Jan. 9, 1987). Therefore, it is an extremely important goal of medical science to produce a vaccine that is prophylactic for individuals identified as being in a high risk group, such as for example, a familial history of breast cancer, and/or treating an individual diagnosed with breast cancer.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for treating an individual suffering from or susceptible to developing HER-2/*neu* positive cancer cells such as breast cancer cells. Non-replicating vectors comprising an oncogene lacking kinase signaling or intracellular domains were used to transduce antigen presenting cells which present antigenic fragments to the immune effector cells resulting in a dramatic decrease of breast cancer cells.

In particular the invention provides for methods and compositions for treating an individual suffering from or susceptible to, tumor growth development. Preferably, the tumor to be treated by the methods and compositions of the invention are directed to HER-2 expressing tumors, such as for example breast cancer.

In a preferred embodiment, a HER-2/*neu* molecule which lacks the intracellular domain, responsible for kinase signaling is cloned into a non-replicating vector such as for example adenoviral vector.

In one aspect of the invention, the tumor antigen and vector is not limited to HER-2 or adenoviral vectors but, can include vectors such as plasmids, viral vectors, naked DNA and the like. Any antigen that is oncogenic may be used such as for example, CEA, K-ras, p53 and the like. The term "compositions" as referred to herein, are intended to include any nucleic acid molecules, including naked DNA, and express a desired tumor antigen.

In a preferred embodiment, the invention provides a composition comprising an effective amount of a truncated HER-2/*neu* molecule in a pharmaceutically accepted carrier. Preferably, the HER-2/*neu* molecule is cloned into a vector which expresses HER-2/ *neu* gene products, fragments or complementary sequences thereof.

In another preferred embodiment, the composition comprises the HER-2/*neu* molecule, fragments or complementary sequences thereof, cloned into a vector which expresses HER-2/ *neu* gene products. Preferably, the vector is an adenovirus shuttle vector and comprises deletions in E1 and E3 early region genes.

In another preferred embodiment, the composition comprises truncated HER-2/*neu* transmembrane domains and/or extracellular domains or fragments and complements thereof. Preferably, the truncated rat HER2/*neu* oncogene, comprising the extracellular and transmembrane domains of HER2/*neu* [rHER2/*neu*(ECDtm)], complements or fragments thereof, are cloned into a shuttle plasmid.

In a preferred embodiment, the composition comprises an adenovirus vector wherein the vector is homologously recombined with the plasmid comprising the extracellular and transmembrane domains of HER2/*neu* by co-transfection into 293 HEK cells. Preferably, the homologously recombined adenovirus vector and the plasmid produce a recombinant adenovirus vector expressing the extracellular and transmembrane domains of HER2/*neu* (Ad.rHER2/*neu*(ECDtm)), or fragments thereof.

In another preferred embodiment, the recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain and at least about 50% to about 99% of the transmembrane domain of HER2/*neu*, or any fragment or combination thereof.

In another preferred embodiment, the composition comprising the recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain of HER2/*neu*, complements or fragments thereof.

In another preferred embodiment, the composition comprising the truncated HER-2/*neu* lacks a HER-2/*neu* intracellular domain and/or the HER-2/*neu* vector does not encode for intracellular domain gene products with kinase activity.

In another preferred embodiment, the composition comprising HER-2/*neu* is encoded by a sequence having at least about 80% up to about 99.9% sequence identity to a molecule identified by SEQ ID NO: 1, complements, species variants or fragments thereof.

In another preferred embodiment, the composition comprising HER-2/*neu* is encoded by a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1, complements, species variants, such as human HER-2/*neu* or fragments thereof.

In another preferred embodiment, the compositions of the invention that are administered to an individual suffering from or susceptible to tumor growth are taken up by antigen presenting cells such as dendritic cells which present the antigen on their cell surface. Dendritic cells are powerful antigen presenting cells can present micro or nanomolar amounts of antigen on their surface. Amounts of peptides required for antigen presentation are well known in the art. Immune effector cells to which antigen are presented to become activated and proliferate. Preferably the immune effector cells are T and B lymphocytes, and in particular CD4+ and CD8+ T cells are activated. Activated T cells recognize, for example, HER-2 tumor cells, resulting in the lysis of such tumor cells. Antigen presentation to the cells of the immune system are also primed to recognize the antigen administered to an individual, thereby, becoming activated when any of these immune effector cells recognize such tumors.

In another preferred embodiment, the compositions of the invention are administered to individuals diagnosed with cancer, in post-operative procedures, as a prophylactic to any individual, especially for example, individuals who are at high risk of developing cancer identified by any means, such as for example a family history.

In another preferred embodiment, the compositions of the invention can be co-administered with adjuvants, such as cytokines, and/or chemotherapy.

In a preferred embodiment, the antigen presenting cells are dendritic cells.

In another preferred embodiment, the compositions of the invention comprise non-replicating adenoviral vectors with deletions in the E1-, and E3-regions and express a HER-2/*neu* molecule that lacks the intracellular domain and/or kinase activity.

In another preferred embodiment, the recombinant adenoviral vector expressing HER-2 comprises HER-2 transmembrane domains and/or extracellular domains. The vector can express the entire HER-2/*neu* molecule, or fragments thereof.

In another preferred embodiment, the activated immune effector cells recognize and kill HER-2/neu positive tumor cells as measured by cytotoxic T cell assays.

In another preferred embodiment, the HER-2/neu is encoded by a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1, more preferably, the HER-2/neu is encoded by a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1, most preferably, the HER-2/neu is encoded by a sequence having at least about 95% sequence identity to a molecule identified by SEQ ID NO: 1.

In one aspect of the invention, any variants, fragments, substantial fragments mutants and the like, of HER-2 or any other tumor antigen can be expressed by a desired vector such as for example, the recombinant adenovirus vector lacking the E1-, E3-early region genes.

In another preferred embodiment peptides are administered to an individual suffering from or susceptible to cancers. Preferably, the peptides of HER-2/*neu* are

encoded by a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1 or fragments thereof, more preferably the peptides of HER-2/*neu* are encoded by a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1 or fragments thereof, even more preferably the peptides of HER-2/*neu* are encoded by a sequence having at least about 95% sequence identity to a molecule identified by SEQ ID NO: 1, or fragments thereof.

In another aspect of the invention, any tumor antigen polypeptide can be administered to an individual diagnosed as suffering from or susceptible to cancers. The polypeptides corresponding to identified tumor antigens can be used to stimulate the cells of the immune system to recognize and lyse tumor cells expressing tumor antigens.

Other aspects of the invention are disclosed *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of the recombinant Adenovirus vectors comprising truncated rat HER2/*neu* oncogenes with extracellular and transmembrane domains [rHER2/*neu*(ECDtm)] or the extracellular domain only [rHER2/*neu*(ECD)]. Ad.EYFP is a vector expressing enhanced yellow fluorescent protein.

Figure 2 is a schematic illustration showing that mice progressively develop cancers in mammary glands between 15 and 25 weeks of age.

Figure 3 is a cell sorter fluorescence graph showing murine bone marrow cells cultured for 8-10 days under conditions described *infra*, generated a CD11c(+), CD11b(+), CD8a(-) myeloid dendritic cells (DC) phenotype.

Figure 4 is a cell sorter fluorescence graph showing on day 8, DC's were infected with Ad.EYFP at varying MOI's. On day 10, 70% and 56% of DC's expressed EYFP at MOI's 30 and 3, respectively.

Figure 5 is a cell sorter fluorescence graph of DC's infected with Ad.rHER2/neu(ECDtm) or Ad.rHER2/neu(ECD) at MOI = 30 showed enhanced expression of the DC maturation markers CD80, CD86, MHC-class I, MHC-class II, and CD40.

Figure 6 is a photograph of the results of a Northern Blot analysis of RNA isolated from DC's infected with Ad.rHER2/*neu*(ECD-tm) or Ad.rHER2/*neu*(ECD), and probed with ^{32}P-labeled fragment of rat Her2/*neu* cDNA showed the expected HER2/*neu* RNA's.

Figure 7 is a graph showing co-culturing of irradiated DC's infected with either of the two Ad.HER2/*neu* vectors with splenic lymphocytes from naïve mice resulted in a greater level of stimulation than culture with uninfected DC's or DC's infected with the Ad.null vector.

Figure 8 is a graph showing the results of three weekly vaccinations of BALB/c-*neu*T transgenic mice with allogeneic DC's infected with Ad.rHER2/*neu*(ECDtm) or Ad.rHER2/*neu*(ECD) beginning at 5-6 weeks of age.

Figure 9 is a graph showing the results of protection against tumors of mice vaccinated with various rAd vectors. Ad.rHER2/*neu*(ECDtm) conferred significant protection over all other treatment groups.

Figure 10 is a schematic representation showing the restriction map of Ad.HER-2/*neu*, an E1-, E3-deleted non-replicating type 5 adenoviral gene transfer vector expressing a truncated HER-2/*neu*.

Figure 11A and 11B are graphs showing the average number of tumors in Her2/*neu* transgenic mice with Ad-Null, Ad-ECD(extracellular domain of Her2/neu), Ad-TM (ECD and transmembrane domain of Her2/neu) or none, starting at the age of 4-6 weeks (Fig. 11A;5 mice per group) or 6-8 weeks (Fig. 11B;3-4 mice per group). Mice were

immunized totally five times with an interval of 3-4 weeks.

Figure 12 are graphs showing the titrating dose of Ad-Her2/*neu* vaccine.

Figure 13A-13F are graphs showing that $CD4^+$ T cells are important for eliciting protective immune responses induced by Ad-Her2/*neu* vaccine. Lymphocytes were depleted with anti-CD4 (Figure 13D), anti-CD8 (Figure 13E) or anti-CD4/anti-CD8 (Figure 13F) treatment (0.5mg/injection, i.p.) at day 0, 1, 2, 5 of immunization with Ad-Her2/neu. Each group was immunized with 10^8 pfu adenovirus twice from the age of 6-8 weeks at an interval of 3 weeks, except for the group in figure 13A (control group; no immunization). Rat Ig (Figure 13C) was used as antibody control.

Figures 14A-14D are graphs showing protection of tumor-injected mice by administration of Ad-Her2/*neu* vaccine. Protection was observed in mice immunized at day –11 (Figure 14C) and day 5 (Figure 14D) of TUBO cell injection but not from naïve (A) or Ad-null group (Figure 14B).

Figure15 is a series of graphs showing the therapeutic effects of Ad-Her2/*neu* vaccine. BALB/c mice were immunized with Ad-Her2/*neu* (10^8 pfu, i.p., once) on days 2, 4, 7, 10 or 15, respectively, after injection with Tubo cells (10^6 cells, s.c.).

Figures 16A-16F are graphs showing results obtained by Ad-Her2/*neu* vaccination. BALB/c mice were subcutaneously injected with TUBO cells (10^6 cell/injection) and were grouped by their tumor sizes after day 17 of TUBO cell injection. Mice having the smallest tumor were used for non-treated-control (Figure 16A) or Ad-null treated group (Figure 16B). Others were immunized once with Ad-Her2/*neu*, 10^7 pfu (Figure 16C) or 10^8 pfu (Figures 16D, 16E, and 16F).

Figures 17A-17E are graphs showing CD4 T cells responses protected against tumor growth in mammary tumor -injected mice model. BALB/c mice were immunized with Ad-TM (i.p.,10^8 pfu/injection, one injection) 7 days before they were injected with TUBO cells (s.c.,10^6 cells/inj.). T cells were depleted with α-CD4 or/and α -CD8 (i.p.,

0.5 mg/inj) from the day of immunization. CD4-depleted group was not protected from tumor growth (Figure 17C) but CD8-depleted group showed tumor prevention (Figure 17D). Rat IgG was used as a control antibody (Figure 17A). Depletion of T lymphocytes were confirmed as less than 1% on day 10 of tumor injection by FACS analysis.

Figures 18A-18C are graphs showing Ad-Her2/*neu* vaccination induced serum antibodies against Her2/neu and prevented tumor development. BALB-neu T mice were immunized with 10^8 pfu of vaccine at the age of 8 weeks and 11 weeks. CD4 T cells (Figure 18B) or CD8 T cells (Figure 18C) were depleted when immunized. Serum antibodies against Her2/neu were detected from tumor-protected groups (Figures 18A and 18C) even after 23 weeks of the final immunization but not from non-protected group (Figure 18B).

Figure s 19A and 19B are flow cytometer plots showing the DC phenotype. Figure 19A shows the expression of various surface markers of bone marrow cells from the femures of BALB/c female mice cultured under GM-CSF for 10 days and examined by flow cytometry analysis. Open curves: isotype controls. Figure 19B shows the results from DC on day 8, infected with Ad.HER2 or Ad.null at MOI 30, harvested on day 10 and examined for the expression level of surface markers.

Figures 20A is a gel showing Northern blotting analysis of HER-2/*neu* expression by Ad.HER2. Figure 20B is a scattergram showing the flow cytomery analysis of HER-2/*neu* expression by Ad.HER2. Open curves: isotype controls.

Figure 21 is a graph showing stimulation of splenocytes proliferation by $DC_{Ad.HER2}$. *Vertical bar*: standard error.

Figures 22A and 22B are graphs showing the antitumor vaccine efficacy of $DC_{Ad.HER2}$ in BALB-*neu*T mice. Vertical *bars*: standard error. (*: $P < 0.0001$)

Figure 23A is a graph showing anti-HER2/neu antibody in sera. Figure 23B shows a scatter gram of IFN-γ producing cells in spleen of vaccinated BALB-*neu*T mice.

Figure 24 are photographs of immunohistochemical stains showing infiltration of CD4+ and CD8+ cells in mammary glands of mice vaccinated with $DC_{Ad.HER2}$.

Figure 25 is a graph showing protection against tumors by $DC_{Ad.HER2}$ vaccination requires CD4+ cells.

Figure 26A and 26B are graphs showing that $DC_{Ad.HER2}$ vaccination is not affected by pre-existing anti-adenovirus immunity.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions and methods for the treatment of tumors. The methods include both, prophylaxis and treatment of individuals suffering from or susceptible to, tumors expressing HER-2 oncogene. The compositions preferably comprise the use of non-replicating vectors encoding a non-signaling modified tumor antigen. In particular, the methods involve the transfer of a gene for the target antigen into dendritic cells. The advantages include: expression of the tumor antigen by the gene transfer vector without requiring detailed knowledge of the peptide epitopes or binding affinities for a particular HLA molecule; the dendritic cells can process the antigen naturally leading to a more effective antigen presentation and an improved immune response of the desired type.

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms that will be used hereinafter.

A "genetic modification" refers to any addition, deletion or disruption to a cell's normal nucleotides. Any method which can achieve the genetic modification of APCs are within the spirit and scope of this invention. Art recognized methods include viral

mediated gene transfer, liposome mediated transfer, transformation, transfection and transduction.

The terms "nucleic acid molecule" or "polynucleotide" will be used interchangeably throughout the specification, unless otherwise specified. As used herein, "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogues thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA--DNA, DNA-RNA and RNA--RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to the "antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

As used herein, the term "fragment or segment", as applied to a nucleic acid sequence, gene or polypeptide, will ordinarily be at least about 5 contiguous nucleic acid bases (for nucleic acid sequence or gene) or amino acids (for polypeptides), typically at least about 10 contiguous nucleic acid bases or amino acids, more typically at least about

20 contiguous nucleic acid bases or amino acids, usually at least about 30 contiguous nucleic acid bases or amino acids, preferably at least about 40 contiguous nucleic acid bases or amino acids, more preferably at least about 50 contiguous nucleic acid bases or amino acids, and even more preferably at least about 60 to 80 or more contiguous nucleic acid bases or amino acids in length. "Overlapping fragments" as used herein, refer to contiguous nucleic acid or peptide fragments which begin at the amino terminal end of a nucleic acid or protein and end at the carboxy terminal end of the nucleic acid or protein. Each nucleic acid or peptide fragment has at least about one contiguous nucleic acid or amino acid position in common with the next nucleic acid or peptide fragment, more preferably at least about three contiguous nucleic acid bases or amino acid positions in common, most preferably at least about ten contiguous nucleic acid bases amino acid positions in common.

A significant "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least 20 nucleotides, more generally at least 23 nucleotides, ordinarily at least 26 nucleotides, more ordinarily at least 29 nucleotides, often at least 32 nucleotides, more often at least 35 nucleotides, typically at least 38 nucleotides, more typically at least 41 nucleotides, usually at least 44 nucleotides, more usually at least 47 nucleotides, preferably at least 50 nucleotides, more preferably at least 53 nucleotides, and in particularly preferred embodiments will be at least 56 or more nucleotides.

A "vector" is a composition which can transduce, transfect, transform or infect a cell, thereby causing the cell to express nucleic acids and/or proteins other than those native to the cell, or in a manner not native to the cell. A cell is "transduced" by a nucleic acid when the nucleic acid is translocated into the cell from the extracellular environment. Any method of transferring a nucleic acid into the cell may be used; the term, unless otherwise indicated, does not imply any particular method of delivering a nucleic acid into a cell. A cell is "transformed" by a nucleic acid when the nucleic acid is transduced into the cell and stably replicated. A vector includes a nucleic acid (ordinarily RNA or DNA) to be expressed by the cell. A vector optionally includes materials to aid

in achieving entry of the nucleic acid into the cell, such as a viral particle, liposome, protein coating or the like. A "cell transduction vector" is a vector which encodes a nucleic acid capable of stable replication and expression in a cell once the nucleic acid is transduced into the cell.

As used herein, the term "downstream" when used in reference to a direction along a nucleotide sequence means in the direction from the 5' to the 3' end. Similarly, the term "upstream" means in the direction from the 3' to the 5' end.

As used herein, the term "gene" means the gene and all currently known variants thereof and any further variants which may be elucidated.

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to a wild type gene. This definition may also include, for example, "allelic", "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. Of particular utility in the invention are variants of wild type target genes. Variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

As used herein, "variant" of polypeptides refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties

(e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological activity may be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR).

The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs,) or single base mutations in which the polynucleotide sequence varies by one base.

The terms, "complementary" or "complements" are used interchangeably throughout and mean that two sequences are complementary when the sequence of one can bind to the sequence of the other in an anti-parallel sense wherein the 3'-end of each sequence binds to the 5'-end of the other sequence and each A, T(U), G, and C of one sequence is then aligned with a T(U), A, C, and G, respectively, of the other sequence. Normally, the complementary sequence of the oligonucleotide has at least 80% or 90%, preferably 95%, most preferably 100%, complementarity to a defined sequence. Preferably, alleles or variants thereof can be identified. A BLAST program also can be employed to assess such sequence identity.

The term "complementary sequence" as it refers to a polynucleotide sequence, relates to the base sequence in another nucleic acid molecule by the base-pairing rules. More particularly, the term or like term refers to the hybridization or base pairing between nucleotides or nucleic acids, such as, for instance, between the two strands of a double stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single stranded nucleic acid to be sequenced or amplified. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single stranded RNA or DNA molecules are said to be substantially complementary when

the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 95% of the nucleotides of the other strand, usually at least about 98%, and more preferably from about 99 % to about 100%. Complementary polynucleotide sequences can be identified by a variety of approaches including use of well-known computer algorithms and software, for example the BLAST program.

A "heterologous" component refers to a component that is introduced into or produced within a different entity from that in which it is naturally located. For example, a polynucleotide derived from one organism and introduced by genetic engineering techniques into a different organism is a heterologous polynucleotide which, if expressed, can encode a heterologous polypeptide. Similarly, a promoter or enhancer that is removed from its native coding sequence and operably linked to a different coding sequence is a heterologous promoter or enhancer. Possible alternative terminology includes "foreign" or "exogenous". A heterologous nucleotide sequence may encode a sequence of amino acids, i.e. a peptide or a polypeptide.

A "promoter," as used herein, refers to a polynucleotide sequence that controls transcription of a gene or coding sequence to which it is operably linked. A large number of promoters, including constitutive, inducible and repressible promoters, from a variety of different sources, are well known in the art and are available as or within cloned polynucleotide sequences (from, e.g., depositories such as the ATCC as well as other commercial or individual sources).

An "enhancer," as used herein, refers to a polynucleotide sequence that enhances transcription of a gene or coding sequence to which it is operably linked. A large number of enhancers, from a variety of different sources are well known in the art and available as or within cloned polynucleotide sequences (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoter sequences (such as the commonly-used CMV promoter) also comprise enhancer sequences.

"Operably linked" refers to a juxtaposition, wherein the components so described are in a relationship permitting them to function in their intended manner. A promoter is operably linked to a coding sequence if the promoter controls transcription of the coding sequence. Although an operably linked promoter is generally located upstream of the coding sequence, it is not necessarily contiguous with it. An enhancer is operably linked to a coding sequence if the enhancer increases transcription of the coding sequence. Operably linked enhancers can be located upstream, within or downstream of coding sequences. A polyadenylation sequence is operably linked to a coding sequence if it is located at the downstream end of the coding sequence such that transcription proceeds through the coding sequence into the polyadenylation sequence.

"Gene delivery," "gene transfer," and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a "transgenes") into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of "naked" polynucleotides (such as electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art and described herein.

As used herein, a "target cell" or "recipient cell" refers to an individual cell or cell which is desired to be, or has been, a recipient of exogenous nucleic acid molecules, polynucleotides and/or proteins. The term is also intended to include progeny of a single cell.

"*In vivo*" gene delivery, gene transfer, gene therapy and the like as used herein, are terms referring to the introduction of a vector comprising an exogenous polynucleotide directly into the body of an organism, such as a human or non-human mammal, whereby the exogenous polynucleotide is introduced to a cell of such organism *in vivo*.

A cell is "transduced" by a nucleic acid when the nucleic acid is translocated into the cell from the extracellular environment. Any method of transferring a nucleic acid into the cell may be used; the term, unless otherwise indicated, does not imply any particular method of delivering a nucleic acid into a cell. A cell is "transformed" by a nucleic acid when the nucleic acid is transduced into the cell and stably replicated. A vector includes a nucleic acid (ordinarily RNA or DNA) to be expressed by the cell. A vector optionally includes materials to aid in achieving entry of the nucleic acid into the cell, such as a viral particle, liposome, protein coating or the like. A "cell transduction vector" is a vector which encodes a nucleic acid capable of stable replication and expression in a cell once the nucleic acid is transduced into the cell.

As used herein, "homologous recombination" means a nucleotide sequence on one vector is homologous to a nucleotide sequence on another vector. Using restriction enzymes to cut the two sequences and ligating the two sequences results in the two vectors combining. For example, the plasmids used herein, such as pDC316, encodes the adenoviral 5'-inverted terminal repeat sequences and can homologously recombine of with the adenoviral backbone plasmid pBGH.lox.deltaE1E3Cre (Microbix™, Toronto, Ontario). Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector (see, *e.g.*, Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors).

Homologous nucleic acid sequences, when compared, exhibit significant sequence identity or similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon

hybridization conditions. The hybridization conditions are described in greater detail below.

"Stringency" is meant the combination of conditions to which nucleic acids are subject that cause the duplex to dissociate, such as temperature, ionic strength, and concentration of additives such as formamide. Conditions that are more likely to cause the duplex to dissociate are called "higher stringency", e.g. higher temperature, lower ionic strength and higher concentration of formamide.

For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, e.g., one will select relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.10 M NaCl at temperatures of about 50° C. to about 70° C.

For certain applications, it is appreciated that lower stringency conditions are required. Under these conditions, hybridization may occur even though the sequences of probe and target strand are not perfectly complementary, but are mismatched at one or more positions. Conditions may be rendered less stringent by increasing salt concentration and decreasing temperature. For example, a medium stringency condition could be provided by about 0.1 to 0.25 M NaCl at temperatures of about 37° C. to about 55° C., while a low stringency condition could be provided by about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20° C. to about 55° C. Thus, hybridization conditions can be readily manipulated depending on the desired results.

The phrase "hybridizing conditions" and its grammatical equivalents, when used with a maintenance time period, indicates subjecting the hybridization reaction admixture, in context of the concentration of the reactants and accompanying reagents in the admixture, to time, temperature, pH conditions sufficient to allow the polynucleotide probe to anneal with the target sequence, typically to form the nucleic acid duplex. Such time, temperature and pH conditions required to accomplish the hybridization depend, as is well known in the art on the length of the polynucleotide probe to be hybridized, the

degree of complementarity between the polynucleotide probe and the target, the guanidine and cytosine content of the polynucleotide, the stringency of the hybridization desired, and the presence of salts or additional reagents in the hybridization reaction admixture as may affect the kinetics of hybridization. Methods for optimizing hybridization conditions for a given hybridization reaction admixture are well known in the art.

As used herein, "substantial homology" in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least 56%, more generally at least 59%, ordinarily at least 62%, more ordinarily at least 65%, often at least 68%, more often at least 71%, typically at least 74%, more typically at least 77%, usually at least 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a fragment derived from SEQ ID NO: 1. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See Kanehisa (1984) *Nuc. Acids Res.* 12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides. The endpoints of the segments may be at many different pair combinations.

An "antigen" is any substance that reacts specifically with antibodies or T lymphocytes (T cells). An "antigen-binding site" is the part of an immunoglobulin

molecule that specifically binds an antigen. Additionally, an antigen-binding site includes any such site on any antigen-binding molecule, including, but not limited to, an MHC molecule or T cell receptor. "Antigen processing" refers to the degradation of an antigen into fragments (*e.g.*, the degradation of a protein into peptides) and the association of one or more of these fragments (*e.g.*, via binding) with MHC molecules for presentation by "antigen-presenting cells" to specific T cells.

"Dendritic cells" (DC) are potent antigen-presenting cells, capable of triggering a robust adaptive immune response *in vivo*. It has been shown that activated, mature DC provide the signals required for T cell activation and proliferation. These signals can be categorized into two types. The first type, which gives specificity to the immune response, is mediated through interaction between the T-cell receptor/CD3 ("TCR/CD3") complex and an antigenic peptide presented by a major histocompatibility complex ("MHC" defined above) class I or II protein on the surface of APCs. The second type of signal, called a co-stimulatory signal, is neither antigen-specific nor MHC- restricted, and can lead to a full proliferation response of T cells and induction of T cell effector functions in the presence of the first type of signals. This two-fold signaling can, therefore, result in a vigorous immune response. As noted *supra*, in most non-avian vertebrates, DC arise from bone marrow-derived precursors. Immature DC are found in the peripheral blood and cord blood and in the thymus. Additional immature populations may be present elsewhere. DC of various stages of maturity are also found in the spleen, lymph nodes, tonsils, and human intestine. Avian DC may also be found in the bursa of Fabricius, a primary immune organ unique to avians. In a preferred embodiment, the dendritic cells of the present invention are mammalian, preferably human, mouse, or rat.

A "co-stimulatory molecule" encompasses any single molecule or combination of molecules which, when acting together with a peptide/MHC complex bound by a T cell receptor on the surface of a T cell, provides a co-stimulatory effect which achieves activation of the T cell that binds the peptide.

A "transgenic animal" is created when gene manipulation is used to modify the germ cells of animals permanently. Typically, foreign genes are placed in the genome by "transgenesis" generating a transgenic organism. An example of a transgenic mouse model of breast cancer is the neuT transgenic mouse [Sacco *et al.*, *Breast Cancer Res. Treat.*, 47:171-180 (1998)] wherein the rat HER-2/*neu* oncogene is under the control of an MMTV promoter.

"Diagnostic" or "diagnosed" means identifying the presence or nature of a pathologic condition. Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of "true positives"). Diseased individuals not detected by the assay are "false negatives." Subjects who are not diseased and who test negative in the assay, are termed "true negatives." The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the "false positive" rate is defined as the proportion of those without the disease who test positive. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

The terms "patient" or "individual" are used interchangeably herein, and is meant a mammalian subject to be treated, with human patients being preferred. In some cases, the methods of the invention find use in experimental animals, in veterinary application, and in the development of animal models for disease, including, but not limited to, rodents including mice, rats, and hamsters; and primates.

As used herein, "fresh tumors" refer to tumors removed from a host by surgical or other means.

As used herein, "proliferative growth disorder, "neoplastic disease," "tumor", "cancer" are used interchangeably as used herein refers to a condition characterized by uncontrolled, abnormal growth of cells. Preferably the cancer to be treated is breast cancer and the abnormal proliferation of cells in the breast can be any cell in the organ. Examples of cancer include but are not limited to, carcinoma, blastoma, and sarcoma. As

used herein, the term "carcinoma" refers to a new growth that arises from epithelium, found in skin or, more commonly, the lining of body organs.

"Treatment" is an intervention performed with the intention of preventing the development or altering the pathology or symptoms of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. "Treatment" may also be specified as palliative care. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. In tumor (e.g., cancer) treatment, a therapeutic agent may directly decrease the pathology of tumor cells, or render the tumor cells more susceptible to treatment by other therapeutic agents, e.g., radiation and/or chemotherapy.

The term "in need of such treatment" as used herein refers to a judgment made by a care giver such as a physician, nurse, or nurse practitioner in the case of humans that a patient requires or would benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a care giver's expertise, but that include the knowledge that the patient is ill, or will be ill, as the result of a condition that is treatable by the compositions of the invention.

"Cells of the immune system" or "immune cells" as used herein, is meant to include any cells of the immune system that may be assayed, including, but not limited to, B lymphocytes, also called B cells, T lymphocytes, also called T cells, natural killer (NK) cells, lymphokine-activated killer (LAK) cells, monocytes, macrophages, neutrophils, granulocytes, mast cells, platelets, Langerhans cells, stem cells, dendritic cells, peripheral blood mononuclear cells, tumor-infiltrating (TIL) cells, gene modified immune cells including hybridomas, drug modified immune cells, and derivatives, precursors or progenitors of the above cell types.

"Immune effector cells" refers to cells capable of binding an antigen and which mediate an immune response. These cells include, but are not limited to, T cells (T lymphocytes), B cells (B lymphocytes), monocytes, macrophages, natural killer (NK)

cells and cytotoxic T lymphocytes (CTLs), for example CTL lines, CTL clones, and CTLs from tumor, inflammatory, or other infiltrates.

"T cells" or "T lymphocytes" are a subset of lymphocytes originating in the thymus and having heterodimeric receptors associated with proteins of the CD3 complex (e.g., a rearranged T cell receptor, the heterodimeric protein on the T cell surfaces responsible for antigen/MHC specificity of the cells). T cell responses may be detected by assays for their effects on other cells (e.g., target cell killing, macrophage, activation, B-cell activation) or for the cytokines they produce.

"CD4" is a cell surface protein important for recognition by the T cell receptor of antigenic peptides bound to MHC class II molecules on the surface of an APC. Upon activation, naïve CD4 T cells differentiate into one of at least two cell types, Th1 cells and Th2 cells, each type being characterized by the cytokines it produces. "Th1 cells" are primarily involved in activating macrophages with respect to cellular immunity and the inflammatory response, whereas "Th2 cells" or "helper T cells" are primarily involved in stimulating B cells to produce antibodies (humoral immunity). CD4 is the receptor for the human immunodeficiency virus (HIV). Effector molecules for Th1 cells include, but are not limited to, IFN-γ, GM-CSF, TNF-α, CD40 ligand, Fas ligand, IL-3, TNF-β, and IL-2. Effector molecules for Th2 cells include, but are not limited to, IL-4, IL-5, CD40 ligand, IL-3, GS-CSF, IL-10, TGF-β, and eotaxin. Activation of the Th1 type cytokine response can suppress the Th2 type cytokine response.

"CD8" is a cell surface protein important for recognition by the T cell receptor of antigenic peptides bound to MHC class I molecules. CD8 T cells usually become "cytotoxic T cells" or "killer T cells" and activate macrophages. Effector molecules include, but are not limited to, perforin, granzymes, Fas ligand, IFN-γ, TNF-α, and TNF-β.

"Activity", "activation" or "augmentation" is the ability of immune cells to respond and exhibit, on a measurable level, an immune function. Measuring the degree

of activation refers to a quantitative assessment of the capacity of immune cells to express enhanced activity when further stimulated as a result of prior activation. The enhanced capacity may result from biochemical changes occurring during the activation process that allow the immune cells to be stimulated to activity in response to low doses of stimulants.

Immune cell activity that may be measured include, but is not limited to, (1) cell proliferation by measuring the cell or DNA replication; (2) enhanced cytokine production, including specific measurements for cytokines, such as IFN-γ, GM-CSF, or TNF-α; (3) cell mediated target killing or lysis; (4) cell differentiation; (5) immunoglobulin production; (6) phenotypic changes; (7) production of chemotactic factors or chemotaxis, meaning the ability to respond to a chemotactin with chemotaxis; (8) immunosuppression, by inhibition of the activity of some other immune cell type; and, (9) apoptosis, which refers to fragmentation of activated immune cells under certain circumstances, as an indication of abnormal activation.

An "adjuvant" is any substance capable of enhancing the immune response to an antigen with which it is mixed. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol, as well as BCG (bacilli Calmette-Guerin) and *Corynabacterium parvum*, which are often used in humans, and ligands of CCR6 and other chemokine receptors.

A "chemokine" is a small cytokine involved in the migration and activation of cells, including phagocytes and lymphocytes, and plays a role in inflammatory responses. Three classes of chemokines have been defined by the arrangement of the conserved cysteine (C) residues of the mature proteins: the CXC or α chemokines that have one amino acid residue separating the first two conserved cysteine residues; the CC or β chemokines in which the first two conserved cysteine residues are adjacent; the C or γ chemokines which lack two (the first and third) of the four conserved cysteine residues.

Within the CXC subfamily, the chemokines can be further divided into two groups. One group of the CXC chemokines have the characteristic three amino acid sequence ELR (glutamic acid-leucine-arginine) motif immediately preceding the first cysteine residue near the amino terminus. A second group of CXC chemokines lack such an ELR domain. The CXC chemokines with the ELR domain (including IL-8, GROα/β/γ, mouse KC, mouse MIP-2, ENA-78, GCP-2, PBP/CTAPIII/ β -TG/NAP-2) act primarily on neutrophils as chemoattractants and activators, inducing neutrophil degranulation with release of myeloperoxidase and other enzymes. The CXC chemokines without the ELR domain (e.g., IP-10/mouse CRG, Mig, PBSF/SDF-1, PF4), the CC chemokines (e.g., MIP-1α, MIP-1 β, RANTES, MCP-1/2/3/4/mouse JE/mouse MARC, eotaxin, I-309/TCA3, HCC-1, C10), and the C chemokines (e.g., lymphotactin), chemoattract and activate monocytes, dendritic cells, T-lymphocytes, natural killer cells, B-lymphocytes, basophils, and eosinophils.

A "cytokine" is a protein made by a cell that affect the behavior of other cells through a "cytokine receptor" on the surface of the cells the cytokine effects. Cytokines manufactured by lymphocytes are sometimes termed "lymphokines." Examples of cytokines include interleukins, interferons and the like.

By "immunologically effective" is meant an amount of the peptide or fragment thereof which is effective to activate an immune response to prevent or treat proliferative cell growth disorders, such as cancer. Obviously, such amounts will vary between species and individuals depending on many factors. For example, higher doses will generally be required for an effective immune response in a human compared with a mouse.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins containing the sequences recited herein. A polypeptide comprising an epitope of a protein containing a sequence as described herein may consist entirely of the epitope, or may contain additional sequences. The additional

sequences may be derived from the native protein or may be heterologous, and such sequences may (but need not) possess immunogenic or antigenic properties.

An "epitope", as used herein, is a portion of a polypeptide that is recognized (i.e., specifically bound) by a B-cell and/or T-cell surface antigen receptor. Epitopes may generally be identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides derived from the native polypeptide for the ability to react with antigen-specific antisera and/or T-cell lines or clones. An epitope of a polypeptide is a portion that reacts with such antisera and/or T-cells at a level that is similar to the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. B-cell and T-cell epitopes may also be predicted via computer analysis. Polypeptides comprising an epitope of a polypeptide that is preferentially expressed in a tumor tissue (with or without additional amino acid sequence) are within the scope of the present invention.

In a preferred embodiment an adenovirus double deletion mutant in the E1 and E3 genes is preferred, although any number of vectors may be used. As an illustrative example, which is not meant to limit or construe the invention in any way, the following is provided. Ad.HER-2/*neu* is a non-replicating E1-, E3-deleted recombinant adenoviral vector expressing a non-functional rat HER-2/ *neu* oncogene (figure 1). It was generated by subcloning of the contiguous rat HER-2/ *neu* (SEQ ID NO: 1, shown in Table 1) extracellular and transmembrane domains into the *Sma*I restriction site of commercially available shuttle plasmid pDC316 (Microbix™, Toronto, Ontario) to generate plasmid pDC316.rHER2/neu(ECDtm). Plasmid pDC316 encodes the adenoviral 5'-inverted terminal repeat, a murine cytomegalovirus (MCMV) promoter and multicloning site. The Ad.HER-2/*neu* vector was generated by homologous recombination of pDC316.rHER2/neu(ECDtm) with the adenoviral backbone plasmid

pBGH.lox.deltaE1E3Cre (Microbix™, Toronto, Ontario). Homologous recombination was accomplished by co-transfection of the two plasmids into 293 HEK cells by calcium phosphate precipitation. Ad.HER-2/*neu* was screened purified and expanded by standard techniques.

These recombinant vectors provide specific anti-tumor effect for patients who have been diagnosed with Her2/*neu*$^{+}$ tumors such as breast, renal, prostate, and other HER2 tumors. However, this antigen is merely an illustrative example and is not meant to be construed as limiting in any way. Examples of other antigens that are useful for treating different types of cancers, include, but not limited to overexpressed or mutated forms of antigens. For example carcinogenic embryonic antigen (CEA) for gastro-intestinal cancers; K-ras for lung, gastrointestinal and bladder cancers; p53 which affects a wide variety of neoplastic growth; SARDT3 in neck and head cancers.

In another preferred embodiment, dendritic cells of an individual, suffering from or susceptible to, for example, breast cancer, are transduced *in vivo* with recombinant adenoviral vectors (rAds) expressing truncated HER-2/*neu* genes. The most important advantage over previous treatments is that dendritic cells from patients had to be isolated, cultured *ex vivo* with a vector, and then re-infusing the cultured dendritic cells into the patient.

The present invention overcomes this deficit in the prior art. Dendritic cells (DC's) are antigen-presenting cells that play a central role in generating immune responses through the processing of antigens and the presentation of antigen peptide epitopes in the context of MHC to interact with the T cell receptor. As mentioned above, prior approaches have used the *ex vivo* loading of autologous DC's with peptide sequences of tumor antigens designed from analyses of peptide specific HLA-binding affinities. These approaches suffer from the problems of having to determine an individual patient's HLA-type and the relative binding affinity of individual peptide sequences of the tumor antigen involving higher costs, tailored treatments and longer treatment times.

In another preferred embodiment, the present invention also provides methods of testing any potential antitumor antigens (e.g., variants of HER-2) in a transgenic mouse assay. Transgenic mice are produced that express a transforming agent (e.g., a growth factor receptor) under the control of a tissue specific promoter. Such mice develop carcinomas that have genetic and pathological features that closely resemble human cancers. For example, in a MMTV-neu transgenic mouse lineage (*neuT*), 100% of the female mice develop mammary adenocarcinomas [Sacco *et al., Gene Therapy,* 2:493-497 (1995); Sacco *et al., Gene Therapy,* 5:383-393 (1998)]. The antigen presentation ability of HER-2 modified dendritic cells to inhibit tumor formation or growth is then ascertained. In one embodiment the size of the tumor is monitored by determining the tumor size and/or weight. The potential tumor antigen encoded by a vector can be administered by a variety of ways including orally, subcutaneously, or intraperitoneally. Generally, at least two groups of animals are used in the assay, with at least one group being a control group which is administered the administration vehicle without the potential tumor antigen e.g. Ad.null. Detailed descriptions of experiments conducted in the *neuT*-transgenic mouse model are in the Examples which follow.

In accordance with the invention, the transduced dendritic cells present antigen to cells of the immune system and activate the immune system to recognize tumor antigen epitopes, such as for example a tumor cell expressing the HER-2 oncogene.

In a preferred embodiment, transduced dendritic cells present antigen, for example, peptide fragments of the HER-2 antigen on their surface. Lymphocytes, specific for the presented antigens, are activated, proliferate and recognize tumor cells expressing the HER-2 antigen. Lymphocytes include, B cells, T helper cells and cytotoxic T cells. Recognition, of any cell expressing antigenic epitopes by the immune cells, results in the destruction of a tumor cell.

While various procedures involving the use of antibodies have been applied in the treatment of tumors, few if any successful attempts using activated cytotoxic T-cells have

been recorded. Theoretically, cytotoxic T-cells would be the preferable means of treating tumors. However, no procedures have been available to specifically activate cytotoxic T-cells. In contrast to antibodies, the T-cell receptors on the surface of CD8 cells cannot recognize foreign antigens directly. Antigen must first be presented to the T cell receptor, such as a dendritic cell.

The presentation of antigen to CD8 T-cells is accomplished by major histocompatibility complex (MHC) molecules of the Class I type. The major histocompatibility complex (MHC) refers to a large genetic locus encoding an extensive family of glycoproteins which play an important role in the immune response. The MHC genes, which are also referred to as the HLA (human leukocyte antigen) complex, are located on chromosome 6 in humans. The molecules encoded by MHC genes are present on cell surfaces and are largely responsible for recognition of tissue transplants as "non-self". Thus, membrane-bound MHC molecules are intimately involved in recognition of antigens by T-cells.

MHC products are grouped into three major classes, referred to as I, II, and III. T-cells that serve mainly as helper cells express CD4 and primarily interact with Class II molecules, whereas CD8-expressing cells, which mostly represent cytotoxic effector cells, interact with Class I molecules.

Class I molecules are membrane glycoproteins with the ability to bind peptides derived primarily from intracellular degradation of endogenous proteins. Complexes of MHC molecules with peptides derived from viral, bacterial and other foreign proteins comprise the ligand that triggers the antigen responsiveness of T-cells. In contrast, complexes of MHC molecules with peptides derived from normal cellular products play a role in "teaching" the T-cells to tolerate self-peptides, in the thymus. Class I molecules do not present entire, intact antigens; rather, they present peptide fragments thereof, "loaded" onto their "peptide binding groove".

The present invention also overcomes problems associated with the sequence of peptides which can be presented and recognized in the context of HLA-peptide complex, by cells of the immune system. HLA haplotypes/allotypes vary from individual to individual and identification of peptides that are presented by certain HLA types, would require determining the individual's HLA type. The HLA type may be determined via standard typing procedures and the peripheral blood lymphocytes (PBLs) purified by Ficoll gradients.

In accordance with the invention, since the peptide, for example HER-2/*neu*, is administered to an antigen presenting cell, for example dendritic cells, each individual's HLA type need not be determined. The antigen presenting cells process and present antigen to the immune system. The immune system will recognize whichever peptide fragment that is presented in the context of HLA molecules by engagement with the receptor of an immune effector cell such as, for example a T cell. Thus, the invention overcomes the problems, cost, and time to identify each individual's HLA type and which peptide sequence would be recognized by that individual's immune system. (See below for a description of antigen presentation and cell activation).

As will be recognized by those in the art, the term "host compatible" or "autologous" cells means cells that are of the same or similar haplotype as that of the subject or "host" to which the cells are administered.

The presentation of Class I MHC molecules bound to peptide alone has generally been ineffective in activating CD8 cells. In nature, the CD8 cells are activated by antigen-presenting cells, such as, for example, dendritic cells, which present not only a peptide-bound Class I MHC molecule, but also a costimulatory molecule. Such costimulatory molecules include B7 which is now recognized to be two subgroups designated as B7.1 and B7.2. It has also been found that cell adhesion molecules such as integrins assist in this process.

Dendritic cells are antigen-presenting cells that are found in all tissues and organs, including the blood. Specifically, dendritic cells present antigens for T lymphocytes, i.e., they process and present antigens, and stimulate responses from naive and memory T cells. In addition to their role in antigen presentation, dendritic cells directly communicate with non-lymph tissue and survey non-lymph for an injury signal (e.g., ischemia, infection, or inflammation) or tumor growth. Once signaled, dendritic cells initiate the immune response by releasing IL-1 which triggers lymphocytes and monocytes. When the CD8 T-cell interacts with an antigen-presenting cell, such as a dendritic cell, having the peptide bound by a Class I MHC and costimulatory molecule, the CD8 T-cell is activated to proliferate and becomes an effector T-cell. See, generally, Janeway and Travers, Immunobiology, published by Current Biology Limited, London (1994), incorporated by reference.

Accordingly, what is needed and which the present invention provides, is a means to activate T-cells so that they proliferate, become cytotoxic for cells expressing the desired antigen, such as for example, HER-2, and maintain memory cells specific for the administered antigen. Thus, the immune system is primed against various tumor epitopes so if spontaneous tumors arise, a pool of primed immune cells exist which become activated to recognize and kill the tumor cells.

A review of the biology of memory T cells may be found in Dutton et al. (1998) *Ann. Rev Immunol* 16:201-23. Memory cells express a different pattern of cell surface markers, and they respond in several ways that are functionally different from those of naive cells. Human memory cells are $CD45RA^-$, $CD45RO^+$. In contrast to naïve cells, memory cells secrete a full range of T cell cytokines.

Chemokines and cytokines also play a powerful role in the development of an immune response. The role of chemokines in leukocyte trafficking is reviewed by Baggiolini (1998) *Nature* 392:565-8, in which it is suggested that migration responses in the complicated trafficking of lymphocytes of different types and degrees of activation will be mediated by chemokines. The use of small molecules to block chemokines is reviewed by Baggiolini and Moser (1997) *J. Exp. Med.* 186:1189-1191.

The role of various specific chemokines in lymphocyte homing has been previously described. For example, Campbell et al. (1998) *Science*, showed that SDF-1 (also called PBSF), 6-C-kine (also called Exodus-2), and MIP-3beta (also called ELC or Exodus-3) induced adhesion of most circulating lymphocytes, including most CD4+ T cells; and MIP-3alpha (also called LARC or Exodus-1) triggered adhesion of memory, but not naïve, $CD4^+$ T cells. Tangemann et al. (1998) *J. Immunol.* 161:6330-7 disclose the role of secondary lymphoid-tissue chemokine (SLC), a high endothelial venule (HEV)-associated chemokine, with the homing of lymphocytes to secondary lymphoid organs. Campbell et al. (1998) *J. Cell Biol* 141(4):1053-9 describe the receptor for SLC as CCR7, and that its ligand, SLC, can trigger rapid integrin-dependent arrest of lymphocytes rolling under physiological shear.

Mature B cells can be measured in immunoassays, for example, by cell surface antigens including CD19 and CD20 with monoclonal antibodies labeled with fluorochromes or enzymes may be used to these antigens. B cells that have differentiated into plasma cells can be enumerated by staining for intracellular immunoglobulins by direct immunofluorescence in fixed smears of cultured cells.

Several different ways, to assess maturity and cell differentiation, are available. For example, one such method is by measuring cell phenotypes. The phenotypes of immune cells and any phenotypic changes can be evaluated by flow cytometry after immunofluorescent staining using monoclonal antibodies that will bind membrane proteins characteristic of various immune cell types.

A second means of assessing cell differentiation is by measuring cell function. This may be done biochemically, by measuring the expression of enzymes, mRNA's, genes, proteins, or other metabolites within the cell, or secreted from the cell. Bioassays may also be used to measure functional cell differentiation or measure specific antibody production directed at a patient's tumor, tumor cell lines or cells from fresh tumors.

Immune cells express a variety of cell surface molecules which can be detected with either monoclonal antibodies or polyclonal antisera. Immune cells that have undergone differentiation or activation can also be enumerated by staining for the

presence of characteristic cell surface proteins by direct immunofluorescence in fixed smears of cultured cells.

In vitro T cell cytotoxic assays are well known to those skilled in the art. A preferred method is to measure cytotoxicity in a 5 hr ^{51}Sodium chromate (^{51}Cr) release assay. In particular, a 20 hr ^{51}Cr-release assay is preferred. Tumor cells, also referred to herein as "target cells" are plated in flat-bottomed microtiter plates and incubated at 37°C overnight. The targets are washed and labeled the next day with ^{51}Cr at 37°C. ^{51}Cr is taken up by the target cells, either by endocytosis or pinocytosis, and is retained in the cytoplasm. The wells containing tumor cells are washed, and then armed or unarmed ATC, referred to as "effector cells" are plated at different E:T ratios and incubated overnight at 37°C. Cytolysis is a measure of the ^{51}Cr released from the target cells into the supernatant due to destruction of the target cells by the effector cells. The microtiter plates are centrifuged at 1000 rpm for 10 minutes and an aliquot of about 50 µl to about 100 µl is removed and the level of radioactivity is measured the next day by a gamma counter and the percent specific lysis calculated.

Percent specific lysis is measured by using the formula:

(^{51}Cr released from the target cells) – (spontaneous ^{51}Cr released from the target cells)/ (maximum ^{51}Cr released from the target cells) - (spontaneous ^{51}Cr released from the target cells) X 100

The spontaneous ^{51}Cr released from the target cells is measured with tumor cells to which no effector cells have been added. Maximum ^{51}Cr released from the target cells is obtained by adding, for example, 1M HCl and represents the total amount of ^{51}Cr present in the cytoplasm of the target cell.

Other means of assaying for T lymphocyte activity is by the mixed lymphocyte reaction described in the examples which follow. Other cytotoxicity assays such as the labeling of target cells with tritiated thymidine (^{3}H-TdR) may also be used. ^{3}H-TdR is taken up by target cells into the nucleus of the cell. Release of ^{3}H-TdR is a measure of cell death by DNA fragmentation. The assay is conducted as above except the incubation

period is at least about 48 hours and 50 µl to about 100 µl of the supernatant is measured by a beta-counter in the presence of at least about 1 ml of scintillation fluid. Calculation of percent specific lysis is performed using the above formula.

In a preferred embodiment, vectors encoding the HER-2 as identified by SEQ ID NO: 1 are presented to cells of the immune system. Preferably, a polypeptide encoded by the nucleic acid molecule is detected by cells of the immune system. The polypeptide is preferably detected in a subject sample, suffering from or susceptible to, any type of breast cancer, or other HER-2 type tumors such as for example renal, prostate, ovarian, gastric and the like.

In accordance with the invention any fragments or variants of the sequence identified by SEQ ID NO: 1 can be used and the polypeptide is encoded by a sequence comprising a sequence identified by SEQ ID NO: 1. Preferably the polypeptide is encoded by a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1. More preferably the polypeptide is encoded by a sequence having at least about 90% sequence identity to a molecule by SEQ ID NO: 1, most preferred, the polypeptide is encoded by a sequence having at least about 95% sequence identity to a molecule identified by SEQ ID NO: 1. Examples of nucleotide sequences, or portions thereof, other than SEQ ID NO: 1, that are useful for encoding HER-2/*neu* polypeptides include those found in the GenBank at reference numbers M11730, X03363, and NM 004448.

In another preferred embodiment, peptides are administered to an individual suffering from or susceptible to cancers. Preferably, the peptides of HER-2/*neu* are encoded by a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1. More preferably, the peptides of HER-2/*neu* are encoded by a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1, most preferably, the peptides of HER-2/*neu* are encoded by a sequence having at least about 95% sequence identity to a molecule identified by SEQ ID NO: 1.

The peptides are administered to an individual suffering from or susceptible to cancers. Definite clinical diagnosis of a particular cancer warrants the administration of the peptides, including the early stages of the disease. Prophylactic applications are warranted in cases where individuals with familial history of disease and predicted to be at risk by reliable prognostic indicators could be treated prophylactically to interdict cancer prior to onset, such as breast cancer; or can be administered post operatively.

Peptide vaccines can be administered in many possible formulations, in pharmacologically acceptable mediums. In the case of a short peptide, the peptide can be conjugated to a carrier, such as KLH, in order to increase its immunogenicity. The vaccine can be administered in conjunction with an adjuvant, various of which are known to those skilled in the art. After initial immunization with the vaccine, a booster can be provided. The vaccines are administered by conventional methods, in dosages which are sufficient to elicit an immunological response, which can be easily determined by those skilled in the art.

Efficacy of the peptide in the context of prevention is judged based on the following criteria: frequency of peptide reactive T cells determined by limiting dilution, proliferation response of peptide reactive T cell lines and clones, cytokine profiles of T cell lines and clones to the desired peptide established from patients. Efficacy is established by decrease in frequency of reactive cells, a reduction in thymidine incorporation with altered peptide compared to native, and a reduction in TNF and IFN-α. Clinical measurements include the relapse rate in one and two year intervals, on a Kaplan-Meier curve, a delay in sustained cancer stage progression reduction in the number and size of tumors including a change in area and volume of T2 images on MRI, and the number and volume of lesions determined by gadolinium enhanced images.

Peptides, variants and fragments thereof, of the present invention may be administered either alone, or as a pharmaceutical composition. Briefly, pharmaceutical compositions of the present invention may comprise one or more of the peptides, in combination with one or more pharmaceutically or physiologically acceptable carriers,

diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like, carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide) and preservatives. In addition, pharmaceutical compositions of the present invention may also contain one or more additional active ingredients, such as, for example, cytokines like β-interferon.

Compositions of the present invention may be formulated for the manner of administration indicated, including for example, for oral, nasal, venous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration. Within other embodiments of the invention, the compositions described herein may be administered as part of a sustained release implant. Within yet other embodiments, compositions of the present invention may be formulized as a lyophilizate, utilizing appropriate excipients which provide stability as a lyophilizate, and subsequent to rehydration.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. Within particularly preferred embodiments of the invention, the peptides, variants, or fragments thereof, or pharmaceutical compositions described herein may be administered at a dosage ranging from about 5 to 50 mg/kg, although appropriate dosages may be determined by clinical trials. Dosages of peptide analogue will be approximately 5-50 mg/kg, but are determined more accurately following trials. Patients may be monitored for therapeutic effectiveness by MRI, and signs of clinical exacerbation, as described above.

MRI can be used to measure active lesions using gadolinium-DTPA-enhanced imaging (McDonald et al. *Ann. Neurol.* 36:14, 1994) or the location and extent of lesions using T_2 -weighted techniques. Briefly, baseline MRIs are obtained. The same imaging plane and patient position are used for each subsequent study. Positioning and imaging

sequences are chosen to maximize lesion detection and facilitate lesion tracing. The same positioning and imaging sequences are used on subsequent studies. The presence, location and extent of MS lesions are determined by radiologists. Areas of lesions are outlined and summed slice by slice for total lesion area. Three analyses may be done: evidence of new lesions, rate of appearance of active lesions, percentage change in lesion area (Paty et al., *Neurology* 43:665, 1993). Improvement due to therapy is established when there is a statistically significant improvement in an individual patient compared to baseline or in a treated group versus a placebo group.

In another aspect of the invention, any tumor antigen polypeptide can be administered to an individual diagnosed as suffering from or susceptible to cancers. The polypeptides corresponding to identified tumor antigens can be used to stimulate the cells of the immune system to recognize and lyse tumor cells expressing tumor antigens, such as for example, CEA, p53, K-ras, and the like.

In one aspect of the invention, the tumor antigen and vector is not limited to HER-2 or adenoviral vectors but, can include vectors such as plasmids, viral vectors, naked DNA and the like. Any antigen that is oncogenic may be used such as for example, CEA, K-ras, p53 and the like.

In a preferred embodiment the polypeptide is expressed at least at a higher level in a patient with cancer as compared to expression levels in normal individuals, preferably the polypeptide is expressed at least about 5 to about 10 fold higher in a patient with cancer as compared to expression in a normal individual. Preferably the cancer is a breast cancer and the subject sample is obtained from a mammalian patient, including a primate such as a human patient.

In yet another aspect, variants of the nucleic acid molecule as identified by SEQ ID NO: 1 can be used to transduce immune cells for the detection and lysing of, for example, HER-2 positive cancers. An "allele" or " variant" is an alternative form of a gene. Of particular utility in the invention are variants of the genes encoding any

potential breast tumor markers identified by the methods of this invention. Variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

The compositions and methods of the present invention also encompass variants of the above polypeptides and nucleic acid sequences encoding such polypeptides. A polypeptide "variant," as used herein, is a polypeptide that differs from the native polypeptide in substitutions and/or modifications, such that the antigenic and/or immunogenic properties of the polypeptide are retained. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antisera and/or T-cells as described above. Nucleic acid variants may contain one or more substitutions, deletions, insertions and/or modifications such that the antigenic and/or immunogenic properties of the encoded polypeptide are retained. One preferred variant of the polypeptides described herein is a variant that contains nucleotide substitutions, deletions, insertions and/or modifications at no more than 20% of the nucleotide positions.

Preferably, but not limited to, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) vat, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. However, any type of substitution is within the scope and embodiments of the invention.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenic or antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

In general, nucleotide sequences encoding all or a portion of the polypeptides described herein may be prepared using any of several techniques. For example, cDNA molecules encoding such polypeptides may be cloned on the basis of the breast tumor-specific expression of the corresponding mRNAs, using differential display PCR. This technique compares the amplified products from RNA template prepared from normal and breast tumor tissue. cDNA may be prepared by reverse transcription of RNA using a random primer, such as for example, $(dT)_{12}$ AG primer. Following amplification of the cDNA using a random primer, a band corresponding to an amplified product specific to the tumor RNA may be cut out from a silver stained gel and subcloned into a suitable vector, such as the adenovirus vector described in the examples which follow. Nucleotide sequences encoding all or a portion of the breast tumor-specific polypeptides disclosed by SEQ ID NO:1 and variants thereof may be amplified from cDNA prepared as described above using any random primers.

Alternatively, a gene encoding a polypeptide as described herein (or a portion thereof) may be amplified from human genomic DNA, or from breast tumor cDNA, via polymerase chain reaction.

In an embodiment of the invention the presence of the one or more nucleic acid molecules is correlated to a sample of a normal subject. The sample is preferably obtained from a mammal suspected of having a proliferative cell growth disorder, in

particular, a breast cancer. Preferably, a nucleic acid molecule that is indicative of a breast cancer comprises a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1, more preferably the nucleic acid molecule comprises a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1, most preferably the nucleic acid molecule comprises a sequence having at least about 95% sequence identity to a molecule identified in any of by SEQ ID NO: 1.

Percent identity and similarity between two sequences (nucleic acid or polypeptide) can be determined using a mathematical algorithm (see, e.g., *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

In another preferred embodiment, HER-2/*neu* fragments and derivatives of the invention are of a sufficient length such that they activate the immune system resulting in the lysing of cancer cells, such as, for example cells expressing HER-2/neu, but these fragments or derivatives do not posses kinase signaling activity. That is these fragments or truncated HER-2/neu fragments lack an intracellular domain. HER-2/*neu* fragments and derivatives, lacking an intracellular domain, thus preferably comprise at least about 90% nucleotides as compared to the sequence identified by SEQ ID NO: 1, usually at least about 80% nucleotides as compared to the sequence identified by SEQ ID NO: 1, more usually at least about 70% nucleotides as compared to the sequence identified by SEQ ID NO: 1, even more typically at least about 40% or 50% nucleotides.

Preferred HER-2/*neu* fragments or derivatives of the invention include those that have at least about 70 percent homology (sequence identity) to the HER-2/*neu* of SEQ ID NO:1, more preferably about 80 percent or more homology to the HER-2/*neu* of SEQ ID

NO:1, still more preferably about 85 to 90 percent or more homology to the HER-2/*neu* of SEQ ID NO:1.

In accordance with the invention the HER-2/*neu* expressed in the vector may code for 10% of the intracellular domain and 100% of the extracellular domain. Conversely, the HER-2/*neu* expressed in the vector may code for 100% of the intracellular domain and 10% of the extracellular domain, or 50% of the intracellular domain and 50% of the extracellular domain, as long as the truncated HER-2/*neu* is able to transduce antigen presenting cells and activate an immune response resulting in the lysis of cancer cells expressing, for example HER-2/neu.

To determine the percent identity of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein nucleic acid "identity" is equivalent to nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at online through the Genetics Computer Group), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70,

or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction with the GAP program include a Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of Meyers and Miller (Comput. Appl. Biosci. 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or version 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The treatment of neoplastic disease or neoplastic cells, refers to an amount of the vectors and/or peptides, described throughout the specification and in the Examples which follow, capable of invoking one or more of the following effects: (1) inhibition, to some extent, of tumor growth, including, (i) slowing down and (ii) complete growth arrest; (2) reduction in the number of tumor cells; (3) maintaining tumor size; (4) reduction in tumor size; (5) inhibition, including (i) reduction, (ii) slowing down or (iii) complete prevention, of tumor cell infiltration into peripheral organs; (6) inhibition, including (i) reduction, (ii) slowing down or (iii) complete prevention, of metastasis; (7) enhancement of anti-tumor immune response, which may result in (i) maintaining tumor size, (ii) reducing tumor size, (iii) slowing the growth of a tumor, (iv) reducing, slowing or preventing invasion or (v) reducing, slowing or preventing metastasis; and/or (8) relief, to some extent, of one or more symptoms associated with the disorder.

Thus in one aspect of the invention any variant, fragment, mutant can be used to transduce immune cells, such as for example dendritic cells, for the treatment of an individual suffering from, or, prophylactically to an individual susceptible to cancer. As discussed above, a preferred use of nucleic acid sequences identified in the present invention, is for the generation of treatments that lyse for example, breast cancer cells. The nucleic acid molecules can be expressed by a vector containing a DNA segment encoding the wild-type, alleles, variants, mutations or fragments of the genes. Mutations

and alleles of the nucleic acid molecules are also preferably used in the construction of a vector for use in treatment. The vector comprising the desired nucleic acid sequence for conferring resistance to, for example, breast cancer, preferably has at least one such nucleic acid sequence. Alternatively, the vector may be comprised of more than one such nucleic acid sequence, or combinations of allelic variants. The vector can also be comprised of cassettes of different allelic variants or wild type nucleic acid molecules.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (herein "Sambrook et al., 1989"); DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization [B. D. Hames & S. J. Higgins eds. (1985)]; Transcription And Translation [B. D. Hames & S. J. Higgins, eds. (1984)]; Animal Cell Culture [R. I. Freshney, ed. (1986)]; Immobilized Cells And Enzymes [IRL Press, (1986)]; B. Perbal, A Practical Guide To Molecular Cloning (1984); F. M. Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

Introducing the genes, fragments or alleles thereof, into an individual can include use of vectors, liposomes, naked DNA, adjuvant-assisted DNA, gene gun, catheters, etc. Vectors include chemical conjugates such as described in WO 93/04701, which has a targeting moiety (e.g. a ligand to a cellular surface receptor), and a nucleic acid binding moiety (e.g. polylysine), viral vector (e.g. a DNA or RNA viral vector), fusion proteins such as described in PCT/US95/02140 (WO 95/22618) which is a fusion protein containing a target moiety (e.g. an antibody specific for a target cell) and a nucleic acid binding moiety (e.g. a protamine), plasmids, phage etc. The vectors can be chromosomal, non-chromosomal or synthetic.

It is a preferred embodiment of this invention that the choice of cells for delivery of the nucleic acid molecules include embryonic stem cells, hematopoietic cells which can be differentiated into monocytes/macrophages using cytokines known in the art and delivery of these cells into an individual. Most preferably, the cells are dendritic cells.

In a preferred embodiment, the vector is a recombinant adenovirus vector with deletions in the E1- and E3-regions of the adenovirus vector as described in detail in the examples which follow. Other vectors may also be used. Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. Viral vectors can be chosen to introduce the genes to cells of choice. Such vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as herpes simplex I virus (HSV) vector (Geller et al., 1995, *J. Neurochem.* 64: 487; Lim et al., 1995, *in* DNA Cloning: Mammalian Systems, D. Glover, ed., Oxford Univ. Press, Oxford, England; Geller et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87: 1149), other adenovirus vectors (LeGal LaSalle et al., 1993, *Science* 259: 988; Davidson et al., 1993, *Nat. Genet.* 3: 219; Yang et al., 1995, *J. Virol.* 69: 2004) and adeno-associated virus vectors (Kaplitt et al., 1994, *Nat. Genet.* 8: 148; Kotin, et al. WO 98/11244 (3/19/1998) and Chiorini, et al WO 99/61601 (12/2/1999)).

Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus vectors result in only short term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The vectors can be introduced by standard techniques, e.g. infection, transfection, transduction or transformation. Examples of modes of gene transfer include for example, naked DNA calcium phosphate precipitation, DEAE dextran, electroporation, protoplast fusion, lipofection, cell microinjection and viral vectors.

The vector can be employed to target essentially any desired target cell. For example, stereotaxic injection can be used to direct the vectors (e.g. adenovirus, HSV) to a desired location. Other methods that can be used include catheters, intravenous, parenteral, intraperitoneal, and subcutaneous injection, and oral or other known routes of administration.

Another preferred method is DNA immunization. DNA immunization employs the subcutaneous injection of a plasmid DNA (pDNA) vector encoding a tumor marker. The pDNA sequence is taken up by antigen presenting cells (APC), preferably by dendritic cells. Once inside the cell, the DNA encoding protein is transcribed and translated and presented to lymphocytes.

Genetic constructs comprise a nucleotide sequence that encodes the nucleic acid sequence of choice and preferably includes an intracellular trafficking sequence operably linked to regulatory elements needed for gene expression.

When taken up by a cell, the genetic construct(s) may remain present in the cell as a functioning extrachromosomal molecule and/or integrate into the cell's chromosomal DNA. DNA may be introduced into cells where it remains as separate genetic material in the form of a plasmid or plasmids. Alternatively, linear DNA which can integrate into the chromosome may be introduced into the cell. When introducing DNA into the cell, reagents which promote DNA integration into chromosomes may be added. DNA sequences which are useful to promote integration may also be included in the DNA molecule. Alternatively, RNA may be administered to the cell. It is also contemplated to provide the genetic construct as a linear minichromosome including a centromere, telomeres and an origin of replication. Gene constructs may remain part of the genetic material in attenuated live microorganisms or recombinant microbial vectors which live in cells. Gene constructs may be part of genomes of recombinant viral vaccines where the genetic material either integrates into the chromosome of the cell or remains extrachromosomal.

Genetic constructs include regulatory elements necessary for gene expression of a nucleic acid molecule. The elements include: a promoter, an initiation codon, a stop codon, and a polyadenylation signal. In addition, enhancers may be required for gene expression of the sequence of choice, for example, the nucleic acid sequences identified by SEQ ID NO: 1, gene, alleles or fragments thereof. It is necessary that these elements be operably linked to the sequence that encodes the desired proteins and that the regulatory elements are operable in the individual to whom they are administered.

Initiation codons and stop codons are generally considered to be part of a nucleotide sequence that encodes the immunogenic target protein. However, it is necessary that these elements are functional in the individual to whom the gene construct is administered. The initiation and termination codons must be in frame with the coding sequence.

Promoters and polyadenylation signals used must be functional within the cells of the individual.

Examples of promoters useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to promoters from Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human Myosin, human Hemoglobin, human muscle creatine and human metallothionein.

Examples of polyadenylation signals useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to SV40 polyadenylation signals and LTR polyadenylation signals.

In addition to the regulatory elements required for DNA expression, other elements may also be included in the DNA molecule. Such additional elements include enhancers. The enhancer may be selected from the group including but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

Genetic constructs can be provided with mammalian origin of replication in order to maintain the construct extrachromosomally and produce multiple copies of the construct in the cell. For example, plasmids pCEP4 and pREP4 from Invitrogen (San Diego, Calif.) contain the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region which produces high copy episomal replication without integration.

In order to maximize protein production, regulatory sequences may be selected which are well suited for gene expression in the cells the construct is administered into. Moreover, codons may be selected which are most efficiently transcribed in the cell. One having ordinary skill in the art can produce DNA constructs which are functional in the cells.

The method of the present invention comprises the steps of administering nucleic acid molecules to tissue of the individual. In some preferred embodiments, the nucleic acid molecules are administered intramuscularly, intranasally, intraperitoneally, subcutaneously, intradermally, or topically or by lavage to mucosal tissue selected from the group consisting of vaginal, rectal, urethral, buccal and sublingual.

In some embodiments, the nucleic acid molecule is delivered to the cells in conjunction with administration of a facilitating agent. Facilitating agents are also referred to as polynucleotide function enhancers or genetic vaccine facilitator agents. Facilitating agents are described in e.g. International Application No. PCT/US94/00899 filed Jan. 26, 1994 and International Application No. PCT/US95/04071 filed Mar. 30, 1995, both incorporated herein by reference. Facilitating agents which are administered

in conjunction with nucleic acid molecules may be administered as a mixture with the nucleic acid molecule or administered separately simultaneously, before or after administration of nucleic acid molecules.

In some preferred embodiments, the genetic constructs of the invention are formulated with or administered in conjunction with a facilitator selected from the group consisting of, for example, benzoic acid esters, anilides, amidines, urethans and the hydrochloride salts thereof such as those of the family of local anesthetics. The facilitating agent is administered prior to, simultaneously with or subsequent to the genetic construct. The facilitating agent and the genetic construct may be formulated in the same composition.

In some embodiments of the invention, the individual is first subject to injection of the facilitator prior to administration of the genetic construct. That is, for example, up to a about a week to ten days prior to administration of the genetic construct, the individual is first injected with the facilitator. In some embodiments, the individual is injected with the facilitator about 1 to 5 days; in some embodiments 24 hours, before or after administration of the genetic construct. Alternatively, if used at all, the facilitator is administered simultaneously, minutes before or after administration of the genetic construct. Accordingly, the facilitator and the genetic construct may be combined to form a single pharmaceutical composition.

In some embodiments, the genetic constructs are administered free of facilitating agents, that is in formulations free from facilitating agents using administration protocols in which the genetic constructions are not administered in conjunction with the administration of facilitating agents.

Nucleic acid molecules which are delivered to cells according to the invention may serve as genetic templates for proteins that function as prophylactic and/or therapeutic immunizing agents. In preferred embodiments, the nucleic acid molecules

comprise the necessary regulatory sequences for transcription and translation of the coding region in the cells of the animal.

In further embodiments of the present invention, the compounds described herein may be used for the immunotherapy of breast cancer. In these embodiments, the compounds (which may be polypeptides, antibodies or nucleic acid molecules) are preferably incorporated into pharmaceutical compositions or vaccines. Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more polypeptides and an immune response enhancer, such as an adjuvant or a liposome (into which the compound is incorporated). Pharmaceutical compositions and vaccines may additionally contain a delivery system, such as biodegradable microspheres which are disclosed, for example, in U.S. Pat. Nos. 4,897,268 and 5,075,109. Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, including one or more separate polypeptides.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention.

Any of a variety of adjuvants may be employed in the vaccines of this invention to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially

available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.), Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.), alum, biodegradable microspheres, monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

In yet another preferred embodiment, autologous dendritic cells can be isolated from a patient, transduced with the vectors described in detail herein, cultured, and re-infused into the patient.

An "isolated" or "purified" population of cells is substantially free of cells and materials with which it is associated in nature. By substantially free or substantially purified APCs is meant at least 50% of the population are APCs, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90% free of non-APCs cells with which they are associated in nature.

Dendritic cells of different maturation stages can be isolated based on the cell surface expression markers. For example, mature dendritic cells are less able to capture new proteins for presentation but are much better at stimulating resting T cells to grow and differentiate. Thus, mature dendritic cells can be of importance. Mature dendritic cells can be identified by their change in morphology; by their nonadherence; and by the presence of various markers. Such markers include, but are not limited to, cell surface markers such as B7.2, CD40, $CD11c^+$, and MHC class II. Alternatively, maturation can be identified by observing or measuring the production of pro-inflammatory cytokines. Dendritic cells can be collected and analyzed using typical cytofluorography and cell sorting techniques and devices, such as a fluorescence-activated cell sorter (FACS). Antibodies specific to cell surface antigens of different stages of dendritic cell maturation are commercially available.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the

spirit and scope of the invention. The following non-limiting examples are illustrative of the invention.

EXAMPLES

In the following Examples, the following Materials and Methods were utilized:

Cell Lines.

TUBO cells were derived from a breast cancer of a BALB-*neu*T transgenic mouse, and N202.1A cells were isolated from a breast cancer of a FVB-*neu*N transgenic mouse (both cell lines were the kind gift of Dr. Patrizia Nanni, University of Bologna, Italy). TUBO and N202.1A constitutively express HER-2/neu and were grown in Dulbecco's modified Eagle's medium (DMEM; BioSource, Rockville, MD) supplemented with 10% heat inactivated fetal bovine serum (FBS; Gemini, Woodland, CA). The HER-2/neu-negative cell line, TS/A (also a gift of Dr. Patrizia Nanni) was established from a spontaneous breast cancer from a BALB/c mouseand was grown in RPMI-1640 (BioSource, Rockville, MD) with 10% heat-inactivated FBS. Human embryonic kidney 293 cells were obtained from ATCC (Manassas, VA) and grown in DMEM with 10% heat-inactivated FBS. All cells were maintained in an incubator at 37°C and 5% CO_2.

Construction of the Vectors

Ad.HER-2/*neu* is a non-replicating E1-, E3-deleted recombinant adenoviral vector expressing a non-functional rat HER-2/ *neu* oncogene (figure 1). E1, E3-deleted adenoviruses expressing HER-2/*neu* (Ad.HER2), and a control vector (Ad.null) were generated using the AdMax® system (Microbix, Toronto, Canada). The vectors were generated by subcloning of the contiguous rat HER-2/ *neu* (SEQ ID NO: 1, shown in Table 1) extracellular and transmembrane domains into the *Sma*I restriction site of commercially available shuttle plasmid pDC316 (Microbix™, Toronto, Ontario) to generate plasmid pDC316.rHER2/*neu*(ECDtm). Plasmid pDC316 encodes the adenoviral 5'-inverted terminal repeat, a murine cytomegalovirus (MCMV) promoter and multicloning site. The Ad.HER-2/*neu* vector was generated by homologous recombination of pDC316.rHER2/*neu*(ECDtm) with the adenoviral backbone plasmid

pBGH.lox.deltaE1E3Cre (Microbix™, Toronto, Ontario). Homologous recombination was accomplished by co-transfection of the two plasmids into 293 HEK cells by calcium phosphate precipitation. Ad.HER-2/*neu* was screened purified and expanded by standard techniques, for example, all viruses were double plaque-isolated, expanded on 293 cells, purified on a CsCl gradient, titrated as plaque forming units (pfu)/mL and stored at –70oC. (See figures 1 and 10). Ad.GFP, a recombinant E1, E3-deleted adenovirus-5 expressing green fluorescent protein was purchased from Quantum Biotechnologies (Toronto, Canada).

Animals.

All animal studies were approved by the Animal Care and Use Committee of the National Cancer Institute. BALB-neuT transgenic mice express the rat HER-2/*neu* oncogene under the control of a chimeric mouse mammary tumor virus promoter. These mice provide an aggressive model of mammary carcinogenesis as the transgene is over expressed in rudimentary mammary glands of 3-week-old mice. Atypical lobular hyperplasia is seen at 6-weeks of age that progresses to multiple *in-situ* carcinomas that enlarge and converge to form rapidly growing, invasive and metastasizing tumors in all ten glands by 25-weeks of age. Female BALB/c mice (5 to 6 weeks old) were obtained from the Division of Cancer Treatment, NCI (Fredrick, MD).

Generation of Bone-Marrow-derived Dendritic Cells (DCs)

Dendritic cells (DCs) were derived from murine bone marrow harvested from the femurs of BALB/c mice. Erythrocytes were lysed with ACK buffer (BioWhittker, Walkersville, MD) and nucleated cells were plated in RPMI-1640 with 10% heat-inactivated FBS (GIBCO-Invitrogen, Grand Island, NY) and 20 ng/mL murine granulocyte/macrophage colony-stimulating factor (mGM-CSF; PeproTec, Rocky Hill, NJ) in Falcon plastic bacteriological dishes (BD Biosciences, San Jose, CA). On days 3, 6 and 8, the cultures were refreshed with 10 mL of media with 20 ng/mL murine GM-CSF. On day 8, non-adherent cells (DC's) were collected by gentle washing and infected with rAd's. The DC's were used for the vaccinations on day 10 after washing three times in phosphate buffered saline (PBS).

Northern Analysis.

Day 8 DC's were infected Ad.HER2 or Ad.null at a mutiplicity of infection (MOI) = 30 pfu/cell. On day 10, total RNA was isolated using Trizol Reagent (GIBCO-Invitrogen, Grand Island, NY) and treated with DNAse I (Invitrogen, Carlsbad, CA). The RNA was separated on a 1.2% formaldehyde gel, transferred to nylon membranes and probed with a ^{32}P-labeled fragment of rat HER-2/*neu* cDNA.

Flow Cytometry.

DC's were incubated with fluorescein isothiocyanate (FITC) or phycoerytherin (PE)-labeled anti-mouse CD11c, CD11b, CD40, CD80, CD86, H-2Kd, I-Ad, CD8a (BD Pharmingen, San Diego, CA) and analyzed on a FACSort flow cytometer (Becton Dickinson, San Jose, CA). Day 10 Ad.HER2 infected DC's were incubated with anti-rat HER-2/neu monoclonal antibody (Oncogene Research, La Jolla, CA) followed by incubation with secondary FITC-labeled rabbit anti-mouse immunoglobulin and analyzed by FACScan. To detect cytoplasmic HER-2/neu, the DC's were permeabilized using Cytofix/Cytoperm (BD Pharmingen, San Diego, CA) and similarly stained.

Mixed Lymphocyte Proliferation Assays.

Dendritic cell cultures were infected with Ad.rHER-2/*neu*(ECDtm), Ad.rHER-2/*neu* (ECD) or Ad.null at multiplicities of infection (m.o.i.) of 30. Splenocytes obtained from 8-10 week old naïve female BALB-*neu*T mice were incubated with irradiated unmodified DC's or DC's infected with either of the rAd vectors. Four days later, ^{3}H-thymidine was added and its incorporation was measured by a beta-counter.

*Antitumor Vaccination of BALB/c-*neuT *Transgenic Mice using recombinant adenoviral vectors and Ad.HER2-modified Dendritic cells.*

BALB/c-*neuT* transgenic mice express the rat HER-2/neu oncogene under the control of the MMTV promoter. These mice progressively develop cancers in all 10 mammary glands between 15 and 25 weeks of age (Figure 2). Five to six week old

BALB/c-*neuT* mice were vaccinated sub-cutaneously on a weekly basis for 3 weeks with 1 x 10^6 dendritic cells that were infected with one of the recombinant adenoviral vectors (rAd).

Groups of 5-6 week old female BALB-*neu*T mice received three weekly subcutaneous (s.c.) injections of 1 x 10^6 DC's infected with Ad.HER2 ($DC_{Ad.HER2}$) or Ad.null ($DC_{Ad.null}$) both at MOI = 30, or unmodified DC. The mice were followed twice weekly for the development of tumors. Mice that were free of tumor at 28 weeks were challenged with a s.c. injection of 1 x 10^5 HER-2/*neu*-expressing TUBO cells. Another group of 3-4 week old female BALB-*neu*T mice were s.c. injected with Ad.null 1 x 10^8 pfu prior to anti-HER-2/neu vaccination and followed for the development of tumors after undergoing vaccination as described above. This group was compared to mice similarly pre-vaccinated with Ad.null and vaccinated against HER-2/neu by direct s.c. injection of Ad.HER2 1 x10^8 pfu weekly for 3 weeks.

In another set of experiments, groups of 5-6 week old BALB/c mice were s.c. injected with Ad.null 1 x 10^8 pfu weekly for 4 weeks. Two weeks after the last injection of Ad.null, groups of mice were vaccinated with either 1 x 10^6 $DC_{Ad.HER2}$ s.c., or directly with 1 x 10^8 pfu Ad.HER2 s.c. weekly for two weeks. One week after the final vaccination, the mice were challenged with 5 x 10^5 TUBO cells and observed for tumor growth. Tumor volumes were calculated using the formula for the estimate of a rotational ellipse.

In another set of experiments, BALB/c mice vaccinated with $DC_{Ad.HER2}$ were challenged with 5 x 10^5 HER-2/*neu* expressing TUBO cells. Animals free of tumor at 37 days were re-challenged with an injection of 5 x 10^5 TUBO cells, or HER-2/*neu*-negative TS/A cells and followed for development of tumor.

Antibody Depletion of Lymphocyte Populations.

Five to six week old female BALB/c mice were intraperitoneally injected daily for three days, then every three days until the mice were challenged with tumor with 200

mg of anti-CD4, or anti-CD8 purified from the supernatants of hybridomas GK1.5 (ATCC) and 2.43 (ATCC), respectively, or with anti-asialo-GM1 (WAKO, Richmond, VA). Five days after initiation of antibody treatment, the mice were vaccinated as described above. One week following the last vaccination, the mice received a challenge of 5×10^5 TUBO cells and were monitored for tumor growth.

Detection of Serum Anti-HER-2/neu Antibodies.

Blood was obtained by retro-orbital venipuncture from 5-6 week old BALB/c-*neu*T mice prior to vaccination and one week after completion of the third vaccination. The blood was allowed to clot and the serum samples from each treatment group were pooled. N202.1A cells (HER-2/*neu*-positive) were used to detect and quantify anti-HER2/neu antibodies. Briefly, 2×10^5 N202.1A cells were incubated with the sera diluted 1:10 in 2% BSA in PBS at 4 °C for one hour. The cells were washed and incubated with FITC-labeled rabbit anti-mouse immunoglobulin antibody (DAKO, Carpanteria, CA) for one hour and submitted to flow cytometry. Ten thousand cells were examined. The specific N202.1A binding potential of the sera was calculated using the following equation: [(%positive cells with test serum)(fluorescence mean)]-[(%positive cells with control serum)(fluorescence mean)] x serum dilution. Pooled sera obtained from untreated age-matched mice were used as controls.

Detection of Serum Anti-Adenovirus Antibodies.

Sera were obtained from the mice pre- and post-vaccination. Titers of anti-adenovirus antibodies were measured.] Briefly, 5×10^4 A549 cells were seeded in a 24-well plate. Sixteen hours later, 10 ml of 2-fold serially diluted heat-inactivated test sera was incubated with 10 ml of Ad.GFP (2.5×10^6 pfu) at room temperature for 1-hour. The mixture was then added to the A549 cell monolayer (MOI = 30). Thirty-six hours later, the A549 cells were trypsinized, washed and analyzed for GFP expression by flow cytometry. Anti-adenovirus antibody titers were assessed as the serum dilution that resulted in 50% inhibition of GFP expression compared to controls.

ELISPOT Assay.

Spleens were removed from mice one week after the last vaccination. Splenocytes were mechanically dissociated and the erythrocytes were lysed with ACK buffer. One hundred thousand spleen cells were plated in a 96-well dish coated with anti-mouse interferon-γ (IFN-γ) antibody (R&D systems, Minneapolis, MN) and incubated for 48 hours at 37 °C. The plates were developed in accordance with the manufacturer's protocol and the spots counted.

Cytokine Secretion Assay.

Ten million spleen cells from vaccinated or control BALB-*neu*T mice were incubated with 1 x 10^6 irradiated TUBO cells for 2 days. IFN-γ secreting cells were detected using Cytokine Secretion Assay kit (Miltenyi, Auburn, CA) in accordance with the manufacturer's protocol. Briefly, re-stimulated cells were harvested and stained with PE-labeled anti-mouse IFN-γ antibody together with PreCP-labeled CD45RA/B220 and FITC-labeled anti-CD4 or FITC-labeled anti-CD8. For the enrichment of the IFN-γ producing cells, the stained cells were incubated with anti-PE antibody conjugated with magnetic beads for 15 min at 4°C. Cells with magnetic beads were captured with a MS column followed by elution with 1% BSA-containing PBS. Eluted cells were incubated with 7-AAD for 10 min and were analyzed on the FACScan gating out the dead cells and CD45R/B220-positive cells. The IFN-γ positive CD4 or CD8 cells were calculated and reported as percentage.

Immunohistochemical Analysis.

Mammary glands from 8-9 week old BALB-neuT mice and TUBO tumors grown in BALB/c mice were removed, frozen in OCT compound (SAKURA-Finetek U.S.A. Inc., Torrance, CA), and sectioned by cryostat. Endogeneous peroxidase activity was blocked with 0.3% hydrogen peroxide followed by blocking of avidin and biotin using the Avidin/Biotin Blocking Kit (Zymed Laboratories Inc., South San Francisco, CA). After washing with PBS, the tissues were stained with either rabbit anti-mouse CD4 (BD Pharmingen) or anti-mouse CD8 (BD Pharmingen). The slides were washed and then incubated with biotinylated anti-rabbit antibody (Vector Laboratory, Burlingame, CA),

stained with Streptavidin-Horseradish Peroxidase (DAKO, Carpinteria, CA) and counterstained with hematoxylin.

Statistical Analysis.

STATView version 5.01 software (SAS Institute, Cary, NC) was used for log-rank survival analysis and comparisons of tumor multiplicity using the Mann-Whitney U test.

Table 1

rat HER2/neu (SEQ ID NO: 1)

atgatcatcatggagctggcggcctggtgccgctgggggttcctcctcgccctcctgcccccccggaatcg
cgggcacccaagtgtgtaccggcacagacatgaagttgcggctccctgccagtcctgagacccacctgg
acatgctccgccacctgtaccagggctgtcaggtagtgcagggcaacttggagcttacctacgtgcctgc
caatgccagcctctcattcctgcaggacatccaggaagttcagggttacatgctcatcgctcacaaccagg
tgaagcgcgtcccactgcaaaggctgcgcatcgtgagagggacccagctctttgaggacaagtatgccc
tggctgtgctagacaaccgagatcctcaggacaatgtcgccgcctccaccccaggcagaaccccagag
gggctgcgggagctgcagcttcgaagtctcacagagatcctgaagggaggagttttgatccgtgggaac
cctcagctctgctaccaggacatggttttgtggaaggacgtcttccgcaagaataaccaactggctcctgtc
gatatagacaccaatcgttcccgggcctgtccaccttgtgcccccgcctgcaaagacaatcactgttgggg
tgagagtccggaagactgtcagatcttgactggcaccatctgtaccagtggttgtgcccggtgcaagggc
cggctgcccactgactgctgccatgagcagtgtgccgcaggctgcacgggccccaagcattctgactgc
ctggcctgcctccacttcaatcatagtggtatctgtgagctgcactgcccagccctcgtcacctacaacaca
gacacctttgagtccatgcacaaccctgagggtcgctacacctttggtgccagctgcgtgaccacctgccc
ctacaactacctgtctacggaagtgggatcctgcactctggtgtgtcccccgaataaccaagaggtcacag
ctgaggacggaacacagcgttgtgagaaatgcagcaagccctgtgctcgagtgtgctatggtctgggcat
ggagcaccttcgaggggcgagggccatcaccagtgacaatgtccaggagtttgatggctgcaagaagat
ctttgggagcctggcattttgccggagagctttgatggggacccctcctccggcattgctccgctgaggc
ctgagcagctccaagtgttcgaaaccctggaggagatcacaggttacctgtacatctcagcatggccaga
cagtctccgtgacctcagtgtcttccagaaccttcgaatcattcggggacggattctccacgatggcgcgta
ctcattgacactgcaaggcctggggatccactcgctggggctgcgctcactgcgggagctgggcagtgg
attggctctgattcaccgcaacgcccatctctgctttgtacacactgtaccttgggaccagctcttccggaac
ccacatcaggccctgctccacagtgggaaccggccggaagaggacttgtgcgtctcgagcggcttggtc
tgtaactcactgtgtgcccacgggcactgctgggggccagggcccacccagtgtgtcaactgcagtcatt
tccttcggggccaggagtgtgtggaggagtgccgagtatggaaggggctcccccgggagtatgtgagt
gacaagcgctgtctgccgtgtcaccccgagtgtcagcctcaaaacagctcagagacctgctttggatcgg
aggctgatcagtgtgcagcctgcgcccactacaaggactcgtcctcctgtgtggctcgctgccccagtgg
tgtgaaaccggacctctcctacatgcccatctggaagtacccggatgaggagggcatatgccagccgtg
ccccatcaactgcacccactcctgtgtggatctggatgaacgaggctgcccagcagagcagagagcca
gcccggtgacattcatcattgcaactgtagagggcgtcctgctgttcctgatcttagtggtggtcgttggaat
cctaatcaaacgaaggagacagaagatccggaagtatacgatgtaa

Example 1: *Dendritic cell Phenotype*

Murine bone marrow cells cultured for 8-10 days under the conditions described above generated a $CD11c^+$, $CD11b^-$, $CDBa^-$ myeloid dendritic cell phenotype as shown in figure 3.

DC generated from 10-day cultures of bone marrow cells with GM-CSF expressed CD11c and CD11b, but lacked CD8a indicating myeloid differentiation (Figure 19 A). The cells also expressed CD80, CD86, CD40, as well as MHC class I and class II. Surface marker expression on DC infected with Ad.HER2 ($DC_{Ad.HER2}$), or Ad.null ($DC_{Ad.null}$) was examined. Compared to uninfected DC, $DC_{Ad.null}$ and $DC_{Ad.HER2}$ expressed higher levels of CD80, CD86, CD40, and MHC classes I and II indicating DC maturation (Figure 19B).

Example 2: *Transduction Efficiency of rAd to Dendritic Cells*

On day 8, dendritic cells were infected with Ad.EYFP at varying m.o.i.'s. On day 10, 70% and 56% of dendritic cells expressed EYFP at m.o.i.'s 30 and 3, respectively, as shown in figure 4.

RNA was also isolated from DC infected with Ad.HER2 or Ad.null for 36 hours and analyzed for HER-2/neu mRNA expression. $DC_{Ad.HER2}$ showed the expected 2.1 kb truncated HER-2/neu mRNA transcript which was not detected in $DC_{Ad.null}$ (Figure 20A). HER-2/neu transcripts were absent in uninfected BALB-*neu*T bone marrow-derived DC, even when analyzed by reverse transcriptase polymerase chain reaction, indicating tissue-specific expression in the transgenics. To confirm expression of HER-2/neu oncoprotein, DC infected with Ad.HER2 or Ad.null were stained at 48 hours with antibody against rat HER-2/neu and examined by flow cytometry, showed HER-2/*neu* expression on the cell surface and in the cytoplasm (Figure 20B). On the other hand, HER-2/neu expression was not seen in $DC_{Ad.null}$.

Example 3: *Phenotypic Change of Dendritic Cells Following rAd Infection.*

Dendritic cells infected with Ad.rHER-2/*neu* (ECDtm) or Ad.rHER-2/*neu* (ECD) at an m.o.i. of 30 showed enhanced expression of the dendritic cell maturation markers CD80, CD86, MHC-class I, MHC-class II and CD40, as shown in figure 5.

Example 4: *Expression of Rat HER-2/neu RNA in Dendritic Cells Infected with HER-2/neu-expressing rAds.*

RNA isolated from dendritic cells infected with Ad.rHER-2/*neu*(ECDtm) or Ad.rHER-2/*neu*(ECD) and probed with ^{32}P-labeled fragment of rat HER-2/*neu* cDNA showed the expected HER-2/neu RNA's, as shown in figure 6.

Example 5: *Infection of Dendritic Cells with Ad.HER-2/neu Stimulated Lymphocyte Proliferation.*

Co-culturing of irradiated dendritic cells infected with either of the two Ad.HER-2/neu vectors with splenic lymphocytes from naïve mice resulted in a greater level of stimulation than cultured with uninfected dendritic cells or dendritic cells infected with the Ad.null vectors as shown in figure 7.

Example 6: *Prophylaxis of Breast Cancer by Transfer of Genetically Modified DC's using Adenoviral-mediated Gene Transfer.*

Three weekly vaccinations of BALB/c-*neu*T transgenic mice with allogeneic DC's infected with Ad.rHER2/*neu*(ECDtm) or Ad.rHER2/*neu*(ECD) beginning at 5-6 weeks of age improved the tumor-free survival of the mice, as shown in Figure 8. The mice in the control groups: DC's + Ad.null, or unmodified DC's alone began to develop tumors by 14 weeks of age and all mice in these groups had developed breast cancer by 23.5 weeks. The earliest animal in the vaccination groups to develop a tumor was at 20 weeks. Vaccination with DC's infected with the rAd expressing the longer HER2/*neu* gene sequence (Ad.rHER2/*neu*(ECDtm) versus Ad.rHer2/*neu*(ECD)) conferred greater protection to the mice. The first mouse developing breast cancer in this group was at 27.5 weeks. In terms of the numbers of tumors occurring in any individual mouse, Ad.rHER2/*neu*(ECDtm) conferred significant protection over all other treatment groups, as shown in Figure 9. At 28 weeks, 82.5% of the mammary glands of animals in the

control groups had developed tumors compared to 60% of the breasts of animals treated with DC's + and the shorter HER2/*neu* oncogene expressing vector, Ad.rHER2/*neu*(ECD). This compared to only 14% of the mammary glands of all the animals receiving DC + Ad.rHER2/*neu*(ECDtm). Vaccination with HER2/*neu*-modified DC's was less effective in preventing tumors in 10-12 week old mice.

Various approaches to vaccination included subcutaneous, intraperitoneal, and intramuscular injection of the vector, as well as vaccination with bone marrow-derived dendritic cells transduced with the Ad.HER-2/*neu* vector. In each of these approaches, vaccination with Ad.HER-2/*neu* resulted in superior disease free survival and a marked reduction in the number of tumors arising compared to unvaccinated control animals, animals vaccinated with an adenovirus expressing truncated HER-2/*neu* gene (ECD only), a recombinant adenovirus encoding no transgene (Ad.null), or in the case of dendritic cells, unmodified dendritic cells. Ad.HER-2/*neu* afforded superior protection to the mice against the development of spontaneous breast cancers and improved survival (figures 8, 9 and 11). Ad.HER-2/neu or its human derivatives can be an effective anticancer vaccine against human breast cancer and other HER-2/neu expressing tumors.

Example 7: *Rejection of Syngeneic Breast Cancer Cell Line (TUBO).*

TUBO is a HER-2/*neu* expressing breast cancer cell line established from a spontaneous tumor from a BALB/c-*neu*T transgenic mouse. TUBO cells are tumorigenic in BALB/c-*neu*T transgenic mice as well as normal BALB/c mice. BALB/c-*neu*T mice vaccinated with DC's + Ad.rHER2/*neu*(ECDtm) and tumor free at 28 weeks were challenged with a subcutaneous injection of 1×10^5 TUBO cells. In three mice free of spontaneous breast cancer, the TUBO cells failed to form tumors. In one mouse carrying spontaneous breast cancers the TUBO cells formed a tumor after a latency (Table 2).

TUBO growth on vaccinated Balb/c-neuT (DC-Ad.rHER2/neu(ECDtm))

spontaneous breast cancer	TUBO growth (day 21)
no tumor	-
no tumor	-
no tumor	-
tumor developed	+

Example 8: *Titration of Ad-Her2/neu vaccine dosage to induce protective immune responses.*

BALB/*neu* T mice were intraperitoneally immunized with 10^5, 10^6, 10^7or 10^8 pfu of Ad-Her2/neu vaccine at the age of 6, 9, 12 and 15 weeks. Tumor development was measured twice a week by palpating mammary glands. Three mice were used for each group. Ad-Null, not expressing Her2/neu protein, was used for control group.

As shown in figure 12, mice immunized with 10^5 or 10^6 pfu dose of vaccine, tumor development was delayed compared to the control group. 10^7 pfu dosage was not sufficient to protect against mammary gland tumor development in BALB-neu T, Her2/*neu*-transgenic mice. In order to induce protective immune responses in mice, 10^8 pfu of Ad-Her2/*neu* vaccine was needed and 3-4 times immunization with the same dosage was effective.

Example 9: *CD4 T cells, but not CD8 T cells, are needed for the protective immune responses.*

Lymphocytes were depleted with anti-CD4 (Figure 13D.), anti-CD8 (Figure 13E.) or anti-CD4/anti-CD8 (Figure 13F.) treatment (0.5mg/injection, i.p.) on days 0, 1, 2, 5 of immunization with Ad-Her2/*neu*. Each group was immunized with 10^8 pfu adenovirus

two times from the age of 6-8 weeks at the interval of 3 weeks, except for the group shown in Figure 13A (control group; no immunization). Rat Ig (Figure 13C.)was used as an antibody control.

Immune responses, shown in figure 13E induced in the absence of $CD8^+$ T cells, were as protective against tumor development as those immune responses shown in figure 13B, induced in the presence of $CD8^+$ T cells. However, protective immune responses could not be elicited without CD4 T cells (Figure 13D.), even though tumor occurrence or growth was retarded compared to control groups, (Figure 13A) or (Figure 13C). When both $CD4^+$ T cells and $CD8^+$ T cells were depleted, this protective immunity was abrogated (Figure 13F.). Therefore, the results indicate that$CD4^+$ T cells are critical for protective immune responses induced by Ad-Her2/*neu* vaccine, but $CD8^+$ T cells are not necessary.

Example 10: *Protection of tumor-injected mice with Ad-Her2/*neu *vaccine ;* in vitro *model.*

BALB/c mice were subcutaneously injected with TUBO cells(10^6cells/injection.), established cell lines from mammary gland tumors in BALB-neu T mice, and intraperitoneally immunized with 10^8 pfu of Ad-Her2/*neu*. Mice were immunized on days -11 (Figure 14C) or +5 (Figure 14D) relative to TUBO injection. Tumor size was measured twice a week. Ad-Null was used as a control. Four mice were used for each group.

Protection was observed in mice immunized at day –11 (Figure 14C) and day 5 (Figure 14D) of TUBO cell injection but not in naïve (Figure 14A) or Ad-null group (Figure 14B). Immunization prior to TUBO injection (Figure 14C) was more effective than after TUBO injection (Figure 14D). Therefore, one immunization with 10^8 pfu of Ad-Her2/*neu* was sufficient to protect BALB/c mice from tumor growth and pre-immunization was more effective.

Example 11: *Therapeutic effects of Ad-Her2/neu vaccine.*

BALB/c mice were immunized with Ad-Her2/neu (10^8 pfu, intra peritoneally, once) on days: 2, 4, 7, 10 or 15 (Figure 15), respectively, after subcutaneous (s.c.) injection with TUBO cells (10^6 cells, s.c.). Mice were arbitrarily distributed to each group after tumor injection. Four mice were used for each group. Tumor size was measured twice a week.

Naïve control mice have tumors larger than 250 mm^2 around day 30 of TUBO injection (see Figure 15). However, immunized mice (day 2, 4, 7, 10 – see Figure 15) were completely cured of TUBO growth around day 30. Mice immunized on day 10 (Figure 15) had tumors which were about 50 mm^2. Moreover mice on day 15 (Figure 15) had tumors, 60-80 mm^2. By immunization Ad-Her2/*neu* vaccine, tumors larger than 100 mm^2 were therapeutically cured.

Example 12: *Therapeutic effects of Ad-Her2/neu vaccine ; Repeat under more severe conditions.*

BALB/c mice were subcutaneously injected with TUBO cells (10^6 cells/injection) and were grouped by their tumor sizes after day 17 of TUBO cell injection. Mice having the smallest tumors (Figure 16A) or Ad-Null treated group (Figure 16B) were used as naïve controls. Others were immunized once with Ad-Her2/*neu*, 10^7 pfu (Figure 16C) or 10^8 pfu (Figures 16D, 16E, and 16F).

With 10^8 pfu of Ad-Her2/*neu* vaccine, tumors as large as 100 mm^2 were cured. Results shown in figure 16F, where tumors developed up to 200 mm^2, the tumors were completely cured around day 20 after vaccination with Ad-Her2/*neu*. Administration of 10^7 pfu Ad-Her2/*neu* was able to induce the therapeutic immune responses but administration of 10^8 pfu Ad-Her2/*neu* was more effective. Ad-Her2/*neu* vaccine induces very potent therapeutic immune responses in mammary tumor injection model of mice.

Example 13 : *$CD4^+$ T cells are needed for protective immune responses against tumor growth in mammary tumor-injected mice model.*

BALB/c mice were immunized with Ad-Her2/*neu* (i.p.,10^8 pfu/injection, once) 7 days before they were injected with TUBO cells (s.c.,10^6 cells/injection). T cells were depleted with anti-CD4 or/and anti-CD8 (i.p., 0.5 mg/injection) from the day of immunization. Tumor size was measured twice a week. Rat IgG was used as a control antibody and 4 mice were used for each group. Depletion of T lymphocytes were confirmed as less than 1% on day 10 of tumor injection by FACS analysis.

The CD4-depleted group was not protected from tumor growth, even though a retardation of tumor growth was observed (Figure 17C). This delay in tumor growth disappeared when $CD8^+$ T cells were also depleted (Figure 17E). However, CD8-depleted mice (Figure 17D) showed tumor prevention as well as mice immunized without T cell depletion (Figure 17B). Therefore, $CD4^+$ T cells but not $CD8^+$ T cells were needed to induce the immune responses against tumor growth in TUBO-injected mice model.

Example 14: *Ad-Her2/neu vaccination induced serum antibodies against Her2/neu by a CD4 T cell-dependent mechanism.*

BALB-neu T mice were immunized with 10^8 pfu of Ad-Her2/*neu* vaccine at the age of 8 weeks and 11 weeks. To examine the role of T cells in inducing protective immune responses against tumor development, $CD4^+$ T cells (Figure 18B.) or $CD8^+$ T cells (Figure 18C) were depleted when immunized. Serum was collected from each mouse at the age of 34 weeks when most naïve BALB-neu T mice had mammary gland tumors, but immunized mice did not. Her2/neu-specific antibodies were detected by FACS using N202.1, a cell line expressing Her2/neu on their surfaces (expressed as MFI, mean fluorescence intensity).

Serum antibodies against Her2/neu were detected from tumor-protected groups (Figures 18A and 18C) even after 23 weeks of the final immunization but not from non-protected group (Figure 18B). CD4-depleted mice couldn't prevent tumor development even though they were immunized with Ad-Her2/neu, nor could they produce antibodies to Her2/neu. However, CD8-depleted mice produced antibodies to Her2/neu and

prevented tumor growth. Thus, Ad-Her2/*neu* vaccination induced serum antibodies against Her2/neu in the presence of $CD4^+$ T cells.

Example 15: DCAd.HER2 Stimulates Proliferation of Naïve Splenocytes.

To examine the stimulatory effect of DC in mixed leukocyte culture, irradiated DCAd.HER2, DCAd.null, or DC were cultured in various ratios with splenocytes from non-immunized BALB-neuT mice and pulsed with [3H]-thymidine. A 3 to 4-fold greater stimulation was seen with DCAd.HER2 compared to DCAd.null or unmodified DC (Figure 21) when the ability of the DC to stimulate naïve BALB-*neu*T splenocytes was examined in mixed lymphocyte culture.

Example 16: Vaccination of BALB-neuT Mice with $DC_{Ad.HER2}$ Prevented Spontaneous Autochthonous Breast Cancers.

BALB-*neu*T develop progressive lesion in mammary glands till all mammary glands are involved in carcinoma as a consequence of rat HER-2/neu expression in mammary gland-specific manner. Female BALB-*neu*T at the age of 5 to 6 weeks old were vaccinated weekly for 3 weeks and monitored for palpable breast cancer. Vaccination with $DC_{Ad.HER2}$ significantly improved tumor-free survival of the mice compared to mice receiving $DC_{Ad.null}$ or unmodified DC (Figure 22A). The median tumor-free survival of mice treated with unmodified DC compared to those receiving $DC_{Ad.null}$ was not different (19.5 vs. 20 weeks, P-value = NS). In contrast, the median tumor-free survival was not reached by 28 weeks in mice receiving DCAd.HER2 ($P < 0.0001$). Furthermore, in the groups of mice treated with unmodified DC or DCAd.null, the onset of the first tumor occurred at 14.5 weeks and all mice developed at least one breast cancer by 23.5 weeks. In the group of mice vaccinated with DCAd.HER2, the first breast lesion occurred at 20 weeks, and 14/21 (66.7%) mice were still free of tumor at 28 weeks. Considering the mean tumor multiplicity, DCAd.HER2 conferred significant protection to the mice compared to other treatments (Figure 22B). At 28 weeks, tumor was palpable in all mammary glands (10/10) of all mice treated with unmodified DC and DCAd.null. In mice vaccinated with DCAd.HER2, the mean tumor multiplicity was only

2.9 ($P < 0.0001$, Mann-Whitney U test). Vaccination of older groups of mice was less effective and the vaccine had little effect in mice that had developed palpable tumors.

Example 17: Vaccination of BALB-neuT Transgenic and BALB/c mice with DCAd.HER2 Inhibited Growth of a Transplantable HER-2/neu-Expressing Carcinoma.

To test if $DC_{Ad.HER2}$-vaccinated BALB-*neu*T mice could successfully reject a challenge with a HER-2/neu-expressing tumor cell line, mice vaccinated at 5-7 weeks of age with $DC_{Ad.HER2}$ and tumor free at 28 weeks ($n = 6$), were challenged with the HER-2/neu-positive TUBO cells established from a breast tumor of a BALB-neuT mouse. A small number ($n = 3$) of mice that had been vaccinated with DC or $DC_{Ad.null}$ were also challenged at 28 weeks. Nine to 11 days after s.c. injection with 1×10^5 TUBO cells, visible tumors were evident in all the mice vaccinated with unmodified DC or $DC_{Ad.null}$. The latency of TUBO tumor emergence was identical to similarly injected unvaccinated BALB/c or BALB-*neu*T mice. In contrast, in BALB-*neu*T mice vaccinated with $DC_{Ad.HER2}$, the appearance of TUBO tumors was delayed (median = 56 days). Notably, in all $DC_{Ad.HER2}$-vaccinated mice, TUBO growth occurred contemporaneous or subsequent to the development of the autochthonous breast cancer, suggesting the loss of immunity to HER-2/*neu* after a period of time (Table 3).

Table 3. $DC_{Ad.HER2}$ vaccination protected BALB-*neu*T mice from challenge with HER-2/*neu*-expressing TUBO breast cancer cells.

Treatment Group	TUBO Tumor (days[b])	Breast Cancer (days[b])
[a]$DC_{Ad.HER2}$	108	80
	56	59
	56	66
	56	25
	28	7
	15	11
$DC_{Ad.null}$	11	n.a.
DC	9	n.a.
DC	10	n.a.

[a]Female BALB-*neu*T mice that were treated with $DC_{Ad.HER2}$ and remained tumor free at the age of 28 weeks old, were challenged with syngeneic TUBO cells and monitored for development of TUBO tumors.
[b]After s.c. injection of 1 x10^5 TUBO cells. n.a.- not applicable.

To examine the specificity of protection derived from vaccination with $DC_{Ad.HER2}$, BALB/c mice (n = 8) were vaccinated with 1 x 106 $DC_{Ad.HER2}$ weekly for two weeks, then were challenged with 5 x 10^5 TUBO cells s.c. one week later. All vaccinated mice remained free of tumor at day 37. These mice were then re-challenged with either 5 x 10^5 HER-2/*neu*-positive TUBO cells (n = 4) or HER-2/*neu*-negative TS/A breast cancer cells (n = 4). All the vaccinated mice injected with TS/A developed tumors by day 8, while all those receiving TUBO cells remained free of disease 121 days later (Table 4).

Table 4. Specific immunity to HER-2/neu expressing tumor by $DC_{Ad.HER2}$

	TUBO challenge*	TS/A challenge*
Tumor free mice / total challenged mice	0/4**	4/4***

*Balb/c mice, which were vaccinated with DC modified by Ad.HER-2/neu and remained tumor free 37 days after TUBO cells injection, were challenged with TUBO cells or TS/A cells subcutaneously. The numbers in table indicate ratio of mice which developed tumor ** 121 days or ***10 days after re-challenge.

Example 18: $DC_{Ad.HER2}$-vaccination Induced Production of Serum Anti-HER-2/neu Antibodies, Interferon (IFN)-γ Secretion by Splenocytes.

For examination of humoral immunity by $DC_{Ad.HER2}$, serum specific binding potential (SBP) of anti-HER-2/neu antibodies in groups of BALB-*neu*T mice before the start of the vaccinations and one week after the final vaccination were assayed. Increase in SBP of anti-HER-2/neu antibodies was detected in the serum of mice vaccinated with $DC_{Ad.HER2}$. A minor increase was seen in mice receiving $DC_{Ad.null}$ and no antibodies were detected in mice vaccinated with unmodified DC (Figure 23A).

IFN-γ production by spleen cells from vaccinated and control mice was examined by ELISPOT assay (Table 5). Production of IFN-γ was increased in the splenocytes of mice vaccinated with $DC_{Ad.HER2}$ compared to mice receiving either $DC_{Ad.null}$ or unmodified DCs alone. These results were confirmed by cytokine secretion assay. When spleen cells were re-stimulated with $DC_{Ad.HER2}$ *in vitro*, $DC_{Ad.HER2}$-vaccinated BALB-*neu*T mice exhibited a 2-fold higher frequency of $CD4^+$ IFN-γ expressing splenic lymphocytes than mice vaccinated with $DC_{Ad.null}$, and 4-fold higher levels than the animals treated with unmodified DCs (Figure 23B). The population of $CD8^+$ IFN-γ producing splenic lymphocytes population was also increased by 2-fold in $DC_{Ad.HER2}$ vaccinated mice over those receiving $DC_{Ad.null}$. $DC_{Ad.HER2}$-vaccinated BALB-*neu*T mice exhibited an approximate 2-fold higher frequency of $CD4^+$ IFN-γ-expressing splenic lymphocytes when re-stimulated *ex vivo* with $DC_{Ad.HER2}$ than did mice vaccinated with $DC_{Ad.null}$, and 9-fold higher levels than animals treated with unmodified DC (Figure 23B).

The numbers of IFN-γ producing $CD8^+$ lymphocytes were also increased 2 to 5-fold in the spleens of $DC_{Ad.HER2}$ vaccinated mice compared to animals receiving $DC_{Ad.null}$ or unmodified DC (Figure 23B).

Table 5. ELISPOT assay for IFN-γ-producing splenocytes

Treatment*	Spots/well
DC	14
DC	38
$DC_{Ad.null}$	74
$DC_{Ad.null}$	57
$DC_{Ad.HER2}$	105
$DC_{Ad.HER2}$	103

*Spleen cells were obtained from BALB-neuT mice one week after the each vaccination.

Example 19: Infiltration of Mammary Glands with CD4+ and CD8+ cells.

Mammary glands were removed from mice at the age of 9 weeks old, one week after the last vaccination. Immunohistochemical staining of mammary glands from BALB-*neu*T mice vaccinated with $DC_{Ad.HER2}$ showed modestly increased numbers of $CD4^+$ and $CD8^+$ cells infiltrating hyperplastic and dysplastic mammary glands. In contrast, the breast tissue of mice receiving the control vaccines showed little cellular infiltrate (Figure 24).

Example 20: CD4+ T cells are Required for Generation of Antitumor Immunity with $DC_{Ad.HER2}$

To explore the mechanism of $DC_{Ad.HER2}$-mediated antitumor vaccination, anti-CD4, anti-CD8 or anti-asialo-GM1 antibodies were administered to groups of normal BALB/c mice prior to and during $DC_{Ad.HER2}$ vaccination. One week after the last vaccination, the mice were injected with 5 x 10^5 TUBO cells. Control animals vaccinated with $DC_{Ad.HER2}$, but not treated with antibodies, were protected from tumor growth (Figre 25). Mice receiving anti-CD8 or anti-asialo GM1 antibodies were also protected by the vaccine; however, mice depleted with anti-CD4 antibody developed tumors indicating that $CD4^+$ T cells are required for generation of antitumor immunity by $DC_{Ad.HER2}$. Treatment with anti-CD4 monoclonal antibodies after completion of the $DC_{Ad.HER2}$ vaccination had no effect on the protection afforded by vaccination.

Example 21: Efficacy of $DC_{Ad.HER2}$ Vaccination is Unaffected by Pre-existing Immunity to Adenovirus.

The humoral response to adenovirus is strong and has been found to impede infection efficiency and gene expression in animal models as well as humans. To examine the effect of pre-existing immunity to adenovirus on the efficacy of $DC_{Ad.HER2}$-vaccination, 3-week-old BALB-*neu*T mice were s.c. injected with Ad.null 1 x 10^8 pfu to generate anti-adenovirus immunity. Three weeks later, the mice were divided into two groups and vaccinated s.c. with the Ad.HER2 vector (1 x 10^8 pfu) itself or with 1 x 10^6 $DC_{Ad.HER2}$. Direct injection of Ad.HER2 is also protective in this model. Four out of seven mice receiving $DC_{Ad.HER2}$ remained free of tumor at 28 weeks. These results were similar to our results in the mice treated with $DC_{Ad.HER2}$ not undergoing pre-vaccination with Ad.null (Figure 26, Figure 22A). In contrast, pre-existing immunity to adenovirus abrogated any protection afforded by direct injection of Ad.HER2. All mice pre-vaccinated with Ad.null and treated with s.c. injections of Ad.HER2 developed breast tumors by 24.5 weeks (Figure 26).

To confirm these results, BALB/c mice were injected weekly for 4 weeks with Ad.null 1 x 10^8 pfu. Two weeks later the mice were treated with s.c. injections of either 1 x 10^8 pfu Ad.HER2 or 1 x 10^6 $DC_{Ad.HER2}$. All mice receiving the Ad.null injections

showed significant increases in serum anti-adenovirus antibody titers, from a mean of less than 1:2 prior to injection to greater than 1:256 one week after the last injection. Untreated mice had a mean baseline titer of less than 1:2 that remained unchanged over 6 weeks. One week later the mice were injected with 5×10^5 TUBO cells. Twenty-one days after challenge, all mice treated directly with Ad.HER2 had developed tumors, whereas six of seven mice receiving $DC_{Ad.HER2}$ remained tumor-free (Figure 26).

All of the references identified hereinabove, are hereby expressly incorporated herein by reference to the extent that they describe, set forth, provide a basis for or enable compositions and/or methods which may be important to the practice of one or more embodiments of the present inventions.

What is claimed:

1. A method for treating a subject suffering from or susceptible to developing HER-2/*neu* positive cancer cells, comprising:

administering to the subject an effective amount of a truncated HER-2/*neu* molecule.

2. The method of claim 1, wherein the HER-2/*neu* molecule is cloned into a vector which expresses HER-2/ *neu* gene products.

3. The method of claim 2, wherein the vector is administered to an immune cell.

4. The method of claim 3, wherein the immune cell is an antigen presenting cell.

5. The method of claim 4, wherein HER-2/ *neu* antigenic fragments are presented to immune effector cells.

6. The method of claim 4, wherein the immune effector cells bind to and kill tumor cells expressing HER-2/*neu*.

7. The method of claim 5, wherein antigen presentation to immune effector cells activates the immune effector cells.

8. The method of claim 6, wherein the immune effector cells are lymphocytes.

9. The method of claim 4, wherein the immune cells are dendritic cells.

10. The method of claim 2, wherein the vector is a viral vector.

11. The method of claim 10, wherein the viral vector is an adenovirus shuttle vector, pBGH.lox.deltaE1E3Cre.

12. The method of claim 11, wherein the adenovirus vector comprises deletions in E1 and E3 early region genes.

13. The method of claim 1, wherein the truncated HER-2/*neu* comprises HER-2/*neu* transmembrane domains and/or extracellular domains.

14. The method of claim 13, wherein the truncated rat HER2/*neu* oncogene, comprising the extracellular and transmembrane domains of HER2/*neu* [rHER2/*neu*(ECDtm)], is cloned into a shuttle plasmid, pDC316.

15. The method of claim 12, wherein the adenovirus vector is homologously recombined with the plasmid comprising the extracellular and transmembrane domains of HER2/*neu* by co-transfection into 293 HEK cells.

16. The method of claim 15, wherein the homologously recombined adenovirus vector and the plasmid produce a recombinant adenovirus vector expressing the extracellular and transmembrane domains of HER2/*neu* (Ad.rHER2/*neu*(ECDtm)).

17. The method of claim 15, wherein recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain and at least about 50% to about 99% of the transmembrane domain of HER2/*neu*, or any combination thereof.

18. The method of claim 13, wherein the truncated rat HER2/*neu* oncogene, comprising the extracellular domains of HER2/*neu* [rHER2/*neu*(ECD)], is cloned into a shuttle plasmid, pDC316.

19. The method of claim 12, wherein the adenovirus vector is homologously recombined with the plasmid comprising the extracellular domains of HER2/*neu* by co-transfection into 293 HEK cells.

20. The method of claim 19, wherein the homologously recombined adenovirus vector and the plasmid produce a recombinant adenovirus vector expressing the extracellular domains of HER2/*neu* (Ad.rHER2/*neu*(ECD)).

21. The method of claim 20, wherein recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain of HER2/*neu*.

22. The method of anyone of claims 2 and 14 through 21, wherein the HER-2/*neu* vector is administered to an individual identified to be at a higher risk of developing HER-2/*neu* tumors than a normal individual.

23. The method of claims 2 and 14 through 21, wherein the HER-2/*neu* vector is administered to an individual diagnosed at an early stage of HER-2/neu positive development.

24. The method of claims 2 and 14 through 21, wherein the HER-2/*neu* vector is administered to an individual at a post-operative stage.

25. The method of claims 2 and 14 through 21, wherein the HER-2/*neu* vector is administered to an individual suffering from or susceptible to developing tumors identified by HER-2/*neu* expression.

26. The method of any one of claim 1 through 25, wherein the immune cells recognize and kill HER-2/*neu* positive tumor cells.

27. The method of claim 1, wherein the truncated HER-2/*neu* lacks a HER-2/*neu* intracellular domain.

28. The method of claim 27, wherein the HER-2/*neu* vector does not encode for intracellular domain gene products with kinase activity.

29. The method of claim 1, wherein the HER-2/*neu* is encoded by a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1.

30. The method of claim 1, wherein the HER-2/*neu* is encoded by a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1.

31. The method of claim 1, wherein the HER-2/*neu* is encoded by a sequence having at least about 95% sequence identity to a molecule identified by SEQ ID NO: 1.

32. The method of any one of claims 1 through 31, wherein the vector encoding HER-2/*neu* is administered in conjunction with cytokines.

33. The method of any one of claims 1 through 31, wherein the vector encoding HER-2/*neu* is administered in conjunction with other chemotherapy.

34. A composition comprising an effective amount of a truncated HER-2/*neu* molecule in a pharmaceutically accepted carrier.

35. The composition of claim 34, wherein the HER-2/*neu* molecule is cloned into a vector which expresses HER-2/ *neu* gene products.

36. The composition of claim 34, wherein the HER-2/*neu* molecule is cloned into a vector which expresses HER-2/ *neu* gene products.

37. The composition of claim 36, wherein the vector is an adenovirus shuttle vector.

38. The composition of claim 37, wherein the adenovirus vector comprises deletions in E1 and E3 early region genes.

39. The composition of claim 34, wherein the truncated HER-2/*neu* comprises HER-2/*neu* transmembrane domains and/or extracellular domains or fragments and complements thereof.

40. The composition of claim 39, wherein the truncated rat HER2/*neu* oncogene, comprising the extracellular and transmembrane domains of HER2/*neu* [rHER2/*neu*(ECDtm)], complements or fragments thereof, are cloned into a shuttle plasmid.

41. The composition of claim 39, wherein the adenovirus vector is homologously recombined with the plasmid comprising the extracellular and transmembrane domains of HER2/*neu* by co-transfection into 293 HEK cells.

42. The composition of claim 41, wherein the homologously recombined adenovirus vector and the plasmid produce a recombinant adenovirus vector expressing the extracellular and transmembrane domains of HER2/*neu* (Ad.rHER2/*neu*(ECDtm)), or fragments thereof.

43. The composition of claim 42, wherein recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain and at least about 50% to about 99% of the transmembrane domain of HER2/*neu*, or any fragment or combination thereof.

44. The composition of claim 43, wherein recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain of HER2/*neu*, complements or fragments thereof.

45. The composition of claim 34, wherein the truncated HER-2/*neu* lacks a HER-2/*neu* intracellular domain.

46. The composition of claim 45, wherein the HER-2/*neu* vector does not encode for intracellular domain gene products with kinase activity.

47. The composition of claim 34, wherein the HER-2/*neu* is encoded by a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1, complements or fragments thereof.

48. The composition of claim 34, wherein the HER-2/*neu* is encoded by a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1, complements or fragments thereof.

49. The composition of claim 34, wherein the HER-2/*neu* is encoded by a sequence having at least about 95% sequence identity to a molecule identified by SEQ ID NO: 1, complements or fragments thereof.

50. The composition of any one of claims 34 through 39, wherein the vector encoding HER-2/*neu* is administered in conjunction with cytokines.

51. The composition of any one of claims 34 through 40, wherein the vector encoding HER-2/*neu* is administered in conjunction with other chemotherapy.

52. The composition of anyone of claims 34 through 51, wherein a HER-2/*neu* vector composition is administered to an individual identified to be at a higher risk of developing HER-2/*neu* tumors than a normal individual.

53. The composition of claims 34 through 51, wherein the HER-2/*neu* vector composition is administered to an individual diagnosed at an early stage of HER-2/neu positive development.

54. The composition of claims 34 through 51, wherein the HER-2/*neu* vector composition is administered to an individual at a post-operative stage.

55. The composition of claims 34 through 51, wherein the HER-2/*neu* vector composition is administered to an individual suffering from or susceptible to developing tumors identified by HER-2/*neu* expression.

56. The composition of claims 34 through 51, wherein the HER-2/*neu* vector composition is administered to an immune cell.

57. The composition of any one of claims 34 through 51, wherein the immune cells recognize and kill HER-2/*neu* positive tumor cells.

58. A method for treating a subject suffering from or susceptible to developing HER-2/neu positive cancer cells, comprising:

administering to the subject an effective amount of a truncated HER-2/neu peptide.

59. The method of claim 58, wherein the peptide is co-administered with an adjuvant and/or cytokine.

60. The method of claim 58, wherein the peptide is co-administered with chemotherapy.

61. A method for treating against HER-2/*neu* positive cancer cells, comprising administering to HER-2/*neu* positive cancer cells an effective amount of a truncated HER-2/*neu* molecule.

62. The method of claim 61, wherein the HER-2/*neu* molecule is cloned into a vector which expresses HER-2/ *neu* gene products.

63. The method of claim 62, wherein the vector is administered to an immune cell.

64. The method of claim 63, wherein the immune cell is an antigen presenting cell.

65. The method of claim 64, wherein HER-2/ *neu* antigenic fragments are presented to immune effector cells.

66. The method of claim 64, wherein antigen presentation to immune effector cells activates the immune effector cells.

67. The method of claim 66, wherein the immune effector cells bind to and kill tumor cells expressing HER-2/*neu*.

68. The method of claim 67, wherein the immune effector cells are lymphocytes.

69. The method of claim 65, wherein the immune cells are dendritic cells.

70. The method of claim 62, wherein the vector is a viral vector.

71. The method of claim 70, wherein the viral vector is an adenovirus shuttle vector, pBGH.lox.deltaE1E3Cre.

72. The method of claim 71, wherein the adenovirus vector comprises deletions in E1 and E3 early region genes.

73. The method of claim 61, wherein the truncated HER-2/*neu* comprises HER-2/*neu* transmembrane domains and/or extracellular domains, or fragments thereof.

74. The method of claim 73, wherein the truncated rat HER2/*neu* oncogene, comprising the extracellular and transmembrane domains of HER2/*neu* [rHER2/*neu*(ECDtm)], or fragments thereof, are cloned into a shuttle plasmid, pDC316.

75. The method of any one of claims 71 through 74, wherein the adenovirus vector is homologously recombined with the plasmid comprising the extracellular and transmembrane domains of HER2/*neu* by co-transfection into 293 HEK cells.

76. The method of claim 75, wherein the homologously recombined adenovirus vector and the plasmid produce a recombinant adenovirus vector expressing the extracellular and transmembrane domains of HER2/*neu* (Ad.rHER2/*neu*(ECDtm)).

77. The method of claim 76, wherein recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain and at least about 50% to about 99% of the transmembrane domain of HER2/*neu*, or any combination thereof.

78. The method of claim 77, wherein recombinant adenovirus vector expresses at least about 50% to about 99% of the extracellular domain of HER2/*neu*.

79. The method of claim 78, wherein the truncated HER-2/*neu* lacks a HER-2/*neu* intracellular domain.

80. The method of claim 79, wherein the HER-2/*neu* vector does not encode intracellular domain gene products with kinase activity.

81. The method of claim 76, wherein the HER-2/*neu* is encoded by a sequence having at least about 80% sequence identity to a molecule identified by SEQ ID NO: 1, or fragments thereof.

82. The method of claim 76, wherein the HER-2/*neu* is encoded by a sequence having at least about 90% sequence identity to a molecule identified by SEQ ID NO: 1, or fragments thereof.

83. The method of claim 76, wherein the HER-2/*neu* is encoded by a sequence having at least about 95% sequence identity to a molecule identified by SEQ ID NO: 1, or fragments thereof.

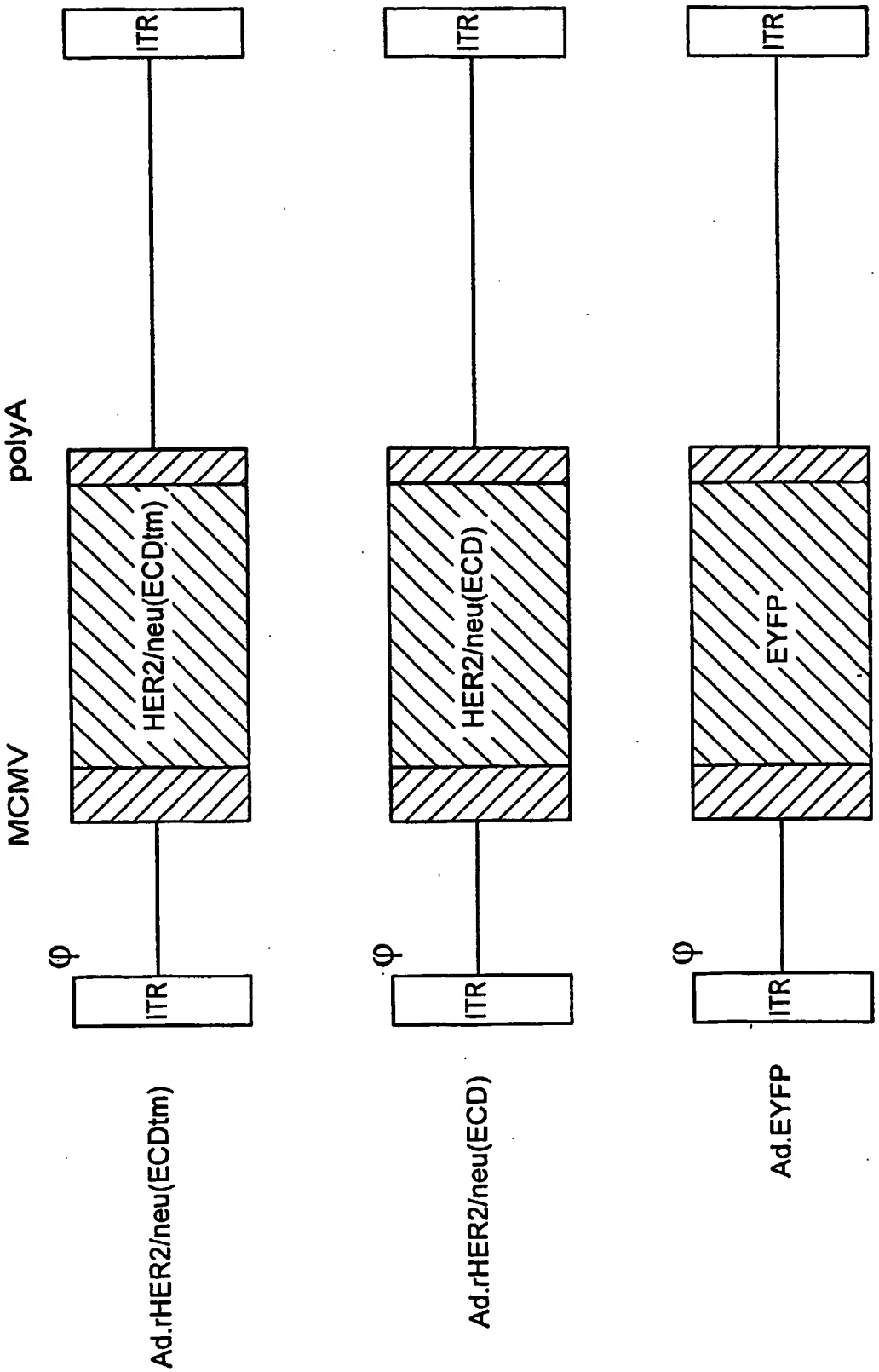
84. The method of any one of claims 61 through 83, wherein a vector encoding HER-2/*neu* is administered to the cells in conjunction with cytokines.

85. The method of any one of claims 61 through 84, wherein a vector encoding HER-2/*neu* is administered to the cells in conjunction with other chemotherapy.

86. A method for treating against HER-2/neu positive cancer cells, comprising administering to HER-2/neu positive cancer cells an effective amount of a truncated HER-2/neu peptide.

87. The method of claim 86, wherein the peptide is co-administered with an adjuvant and/or cytokine.

88. The method of claim 86, wherein the peptide is co-administered with chemotherapy.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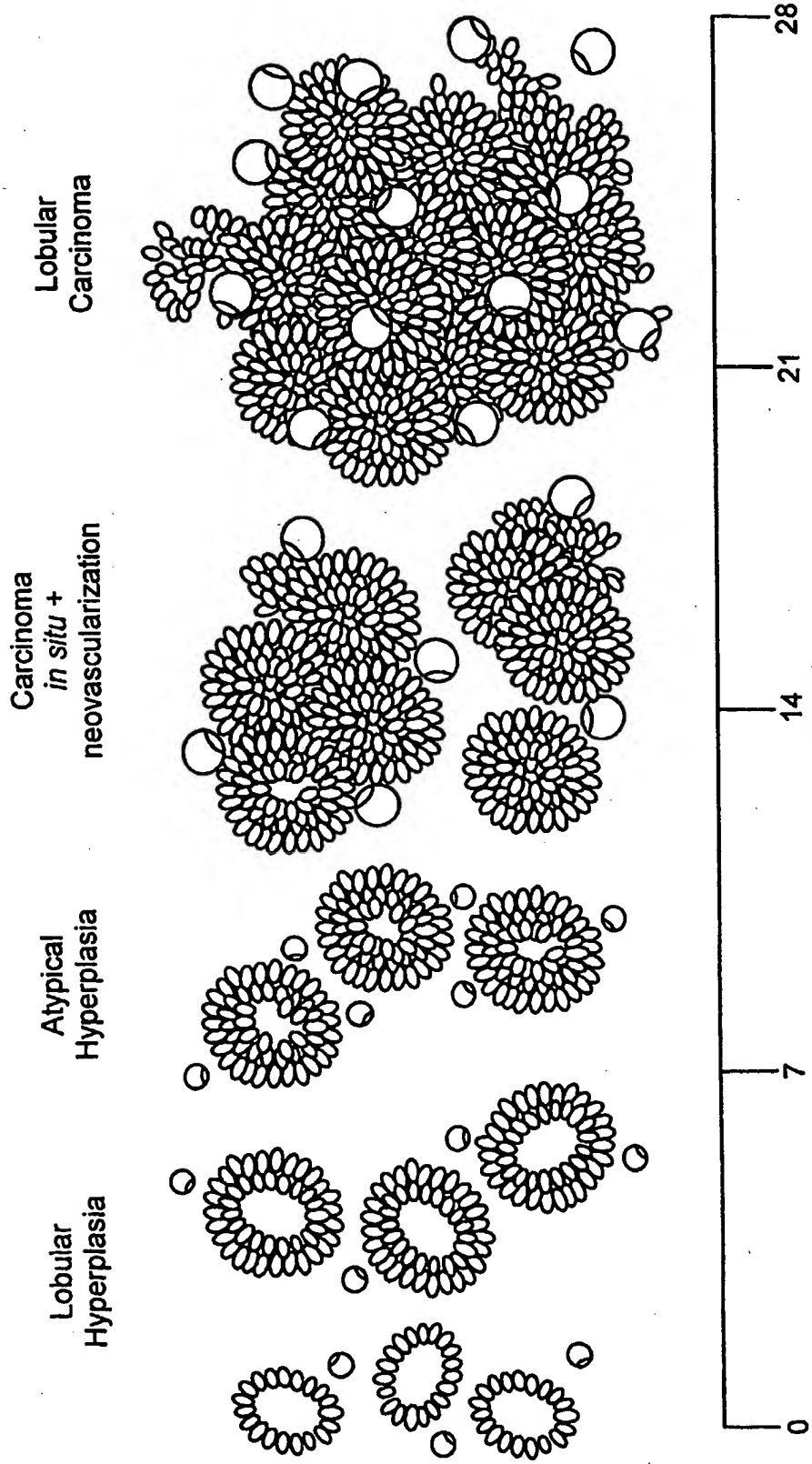
EYFP
φ
ITR
Ad.EYFP

FIG. 1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Carcinoma in situ + neovascularization
Lobular Carcinoma
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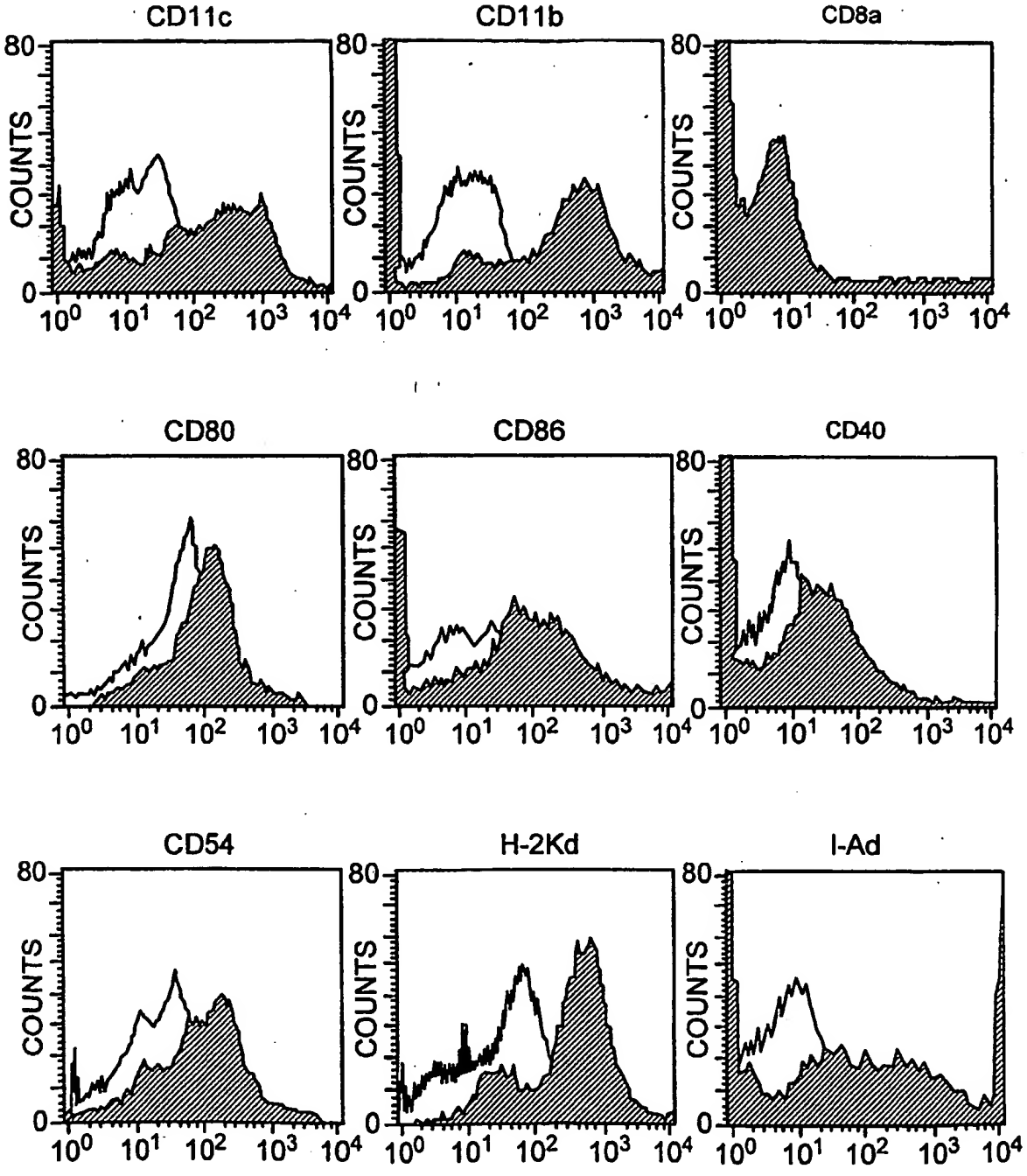
7
14
WEEK
21
28

FIG. 2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CD8a
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I-Ad
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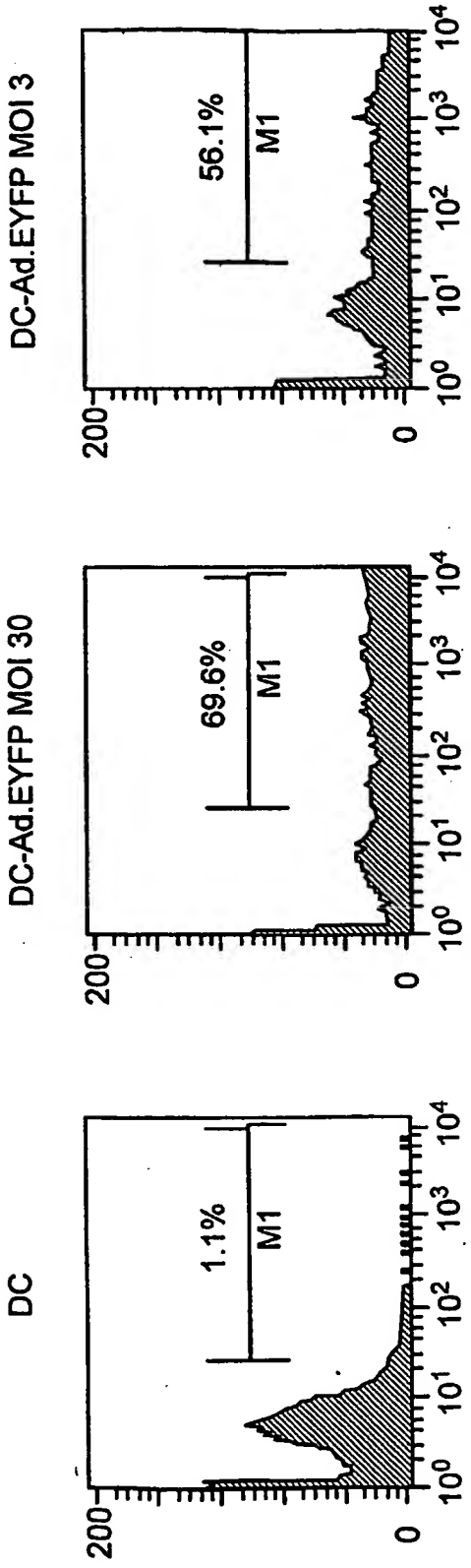
FIG. 3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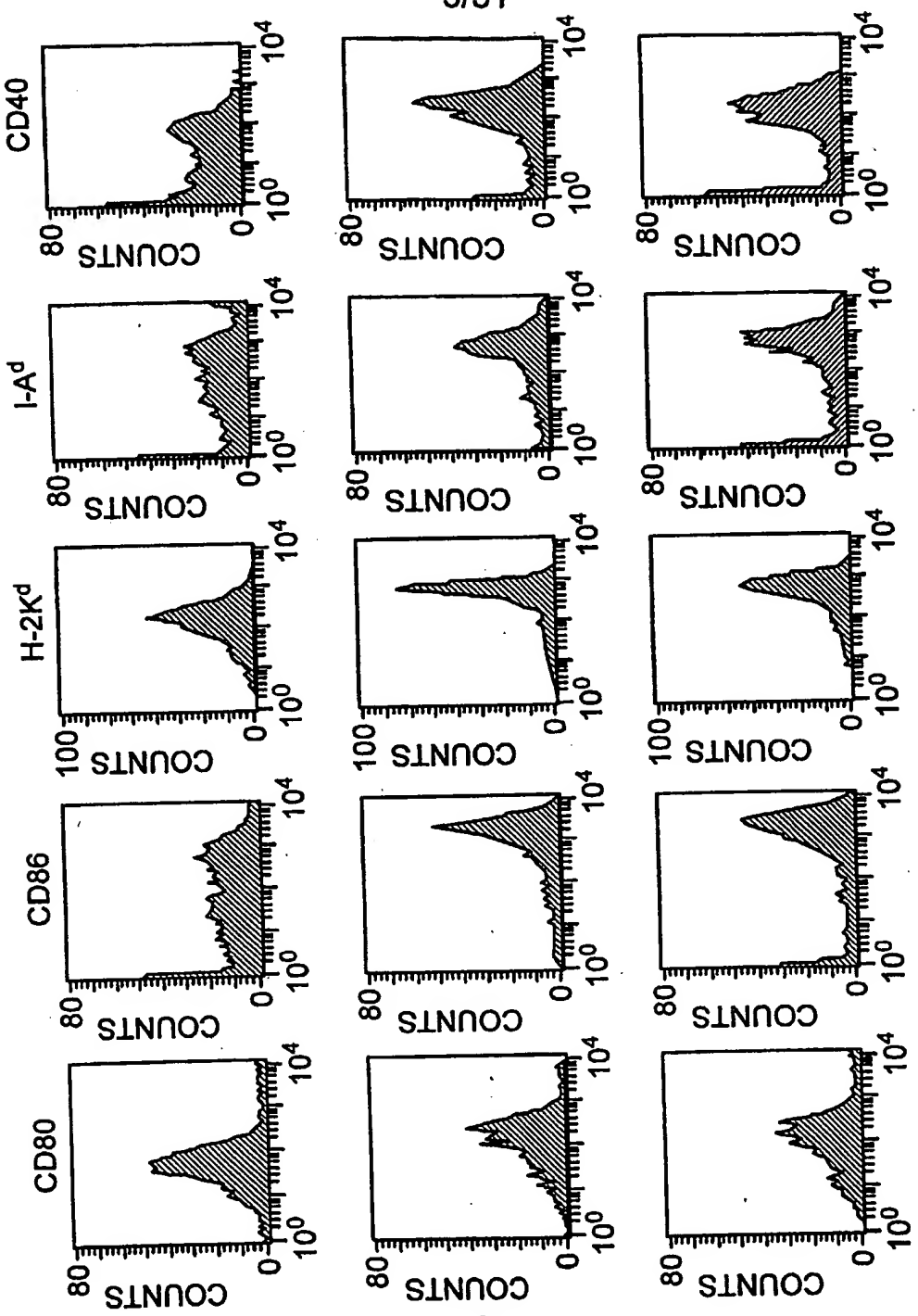
M1
DC-Ad.EYFP MOI 30
69.6%
M1
DC-Ad.EYFP MOI 3
56.1%
M1
200
0
10^0
10^1
10^2
10^3
10^4

FIG. 4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CD40
COUNTS
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DC-Ad.rHER2/neu(ECD)

FIG. 5

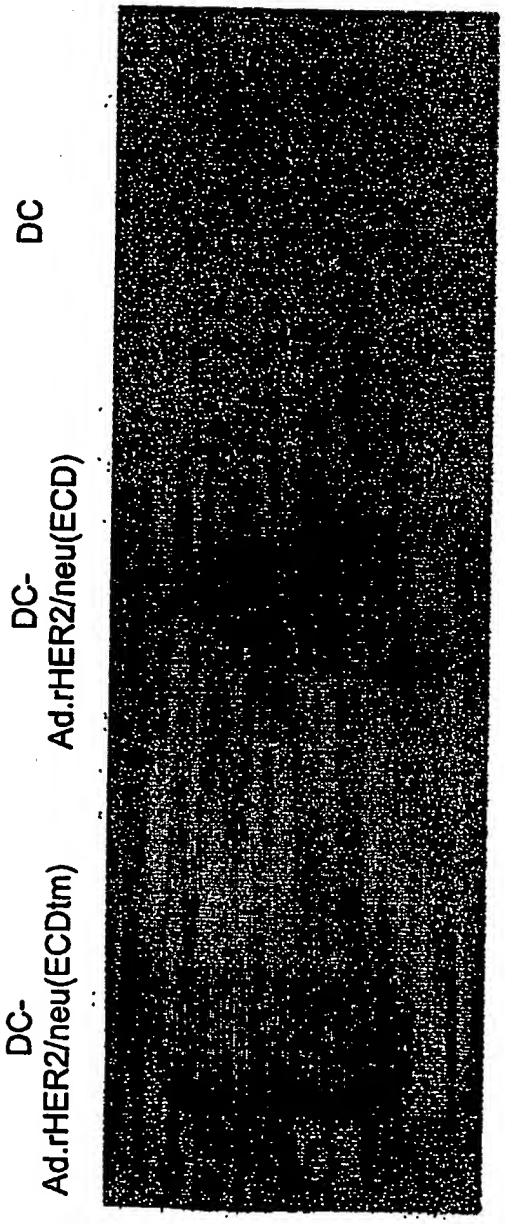

FIG. 6

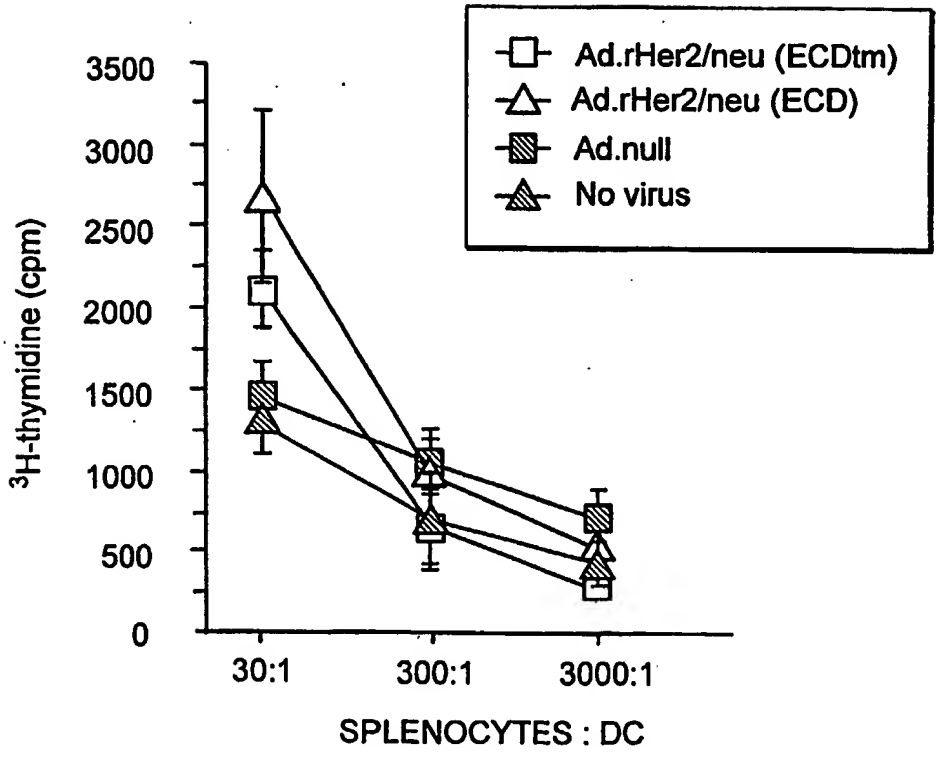
Ad.rHer2/neu (ECDtm)
Ad.rHer2/neu (ECD)
Ad.null
No virus
3500
3000
2500
2000
1500
1000
500
0
^{3}H-thymidine (cpm)
30:1
300:1
3000:1
SPLENOCYTES : DC

FIG. 7

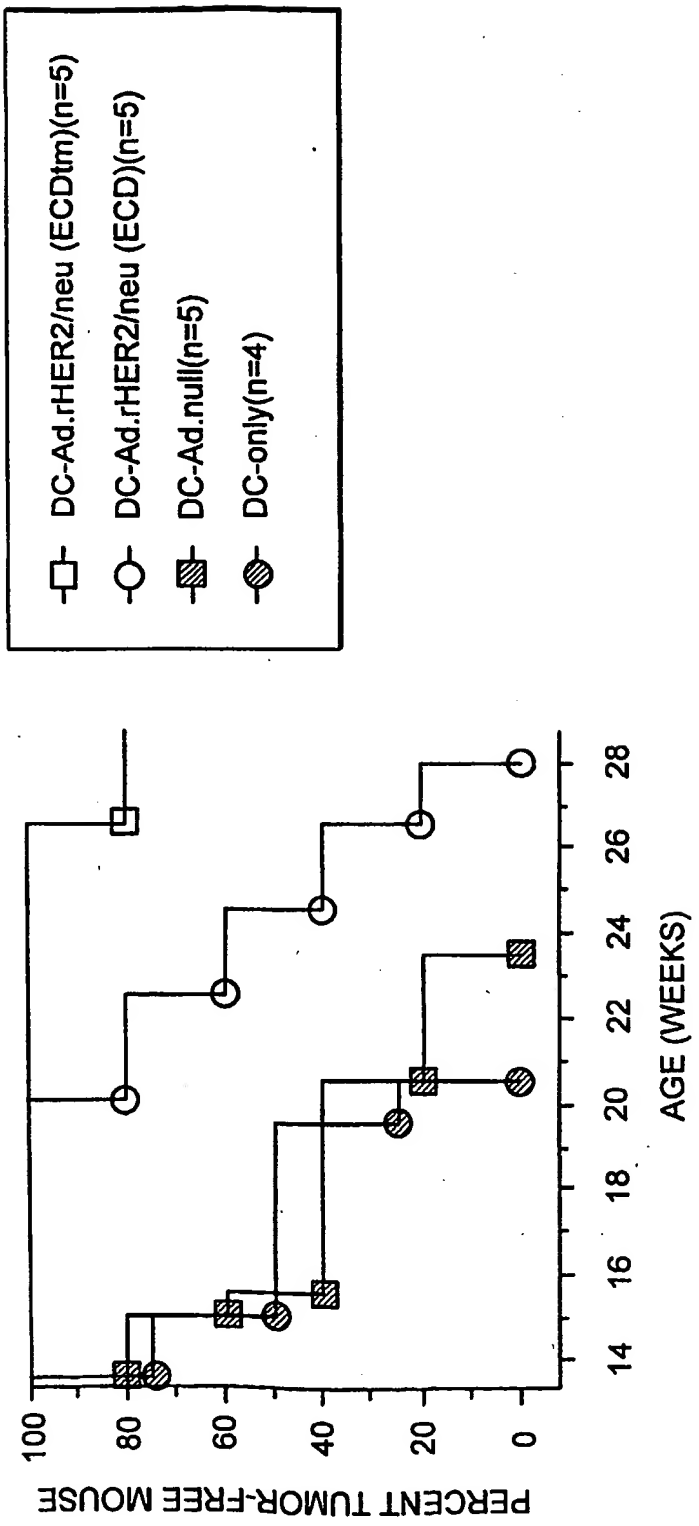
DC-Ad.rHER2/neu (ECDtm)(n=5)
DC-Ad.rHER2/neu (ECD)(n=5)
DC-Ad.null(n=5)
DC-only(n=4)
PERCENT TUMOR-FREE MOUSE
100
80
60
40
20
0
14
16
18
20
22
24
26
28
AGE (WEEKS)

FIG. 8

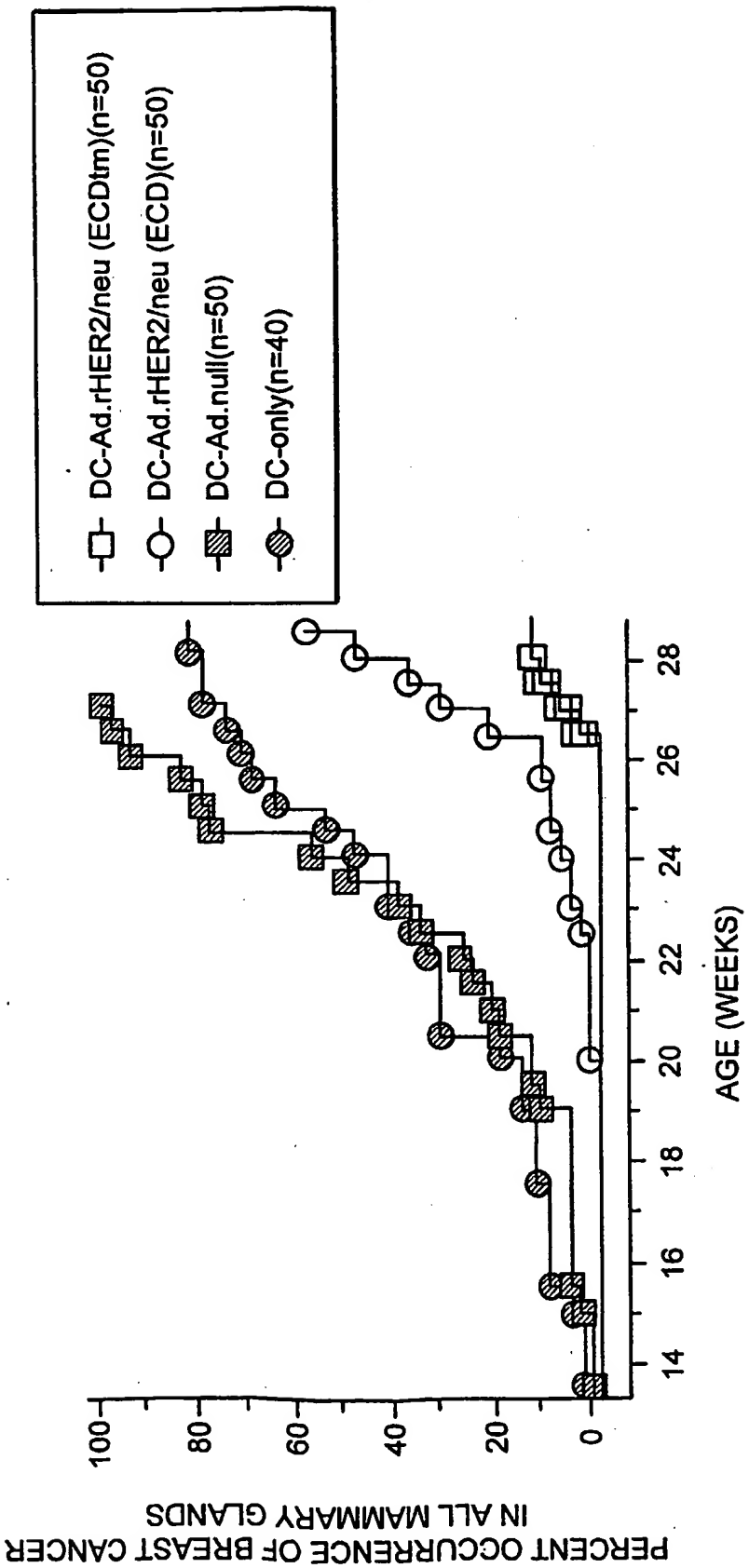
DC-Ad.rHER2/neu (ECDtm)(n=50)
DC-Ad.rHER2/neu (ECD)(n=50)
DC-Ad.null(n=50)
DC-only(n=40)
PERCENT OCCURRENCE OF BREAST CANCER IN ALL MAMMARY GLANDS
100
80
60
40
20
0
14
16
18
20
22
24
26
28
AGE (WEEKS)

FIG. 9

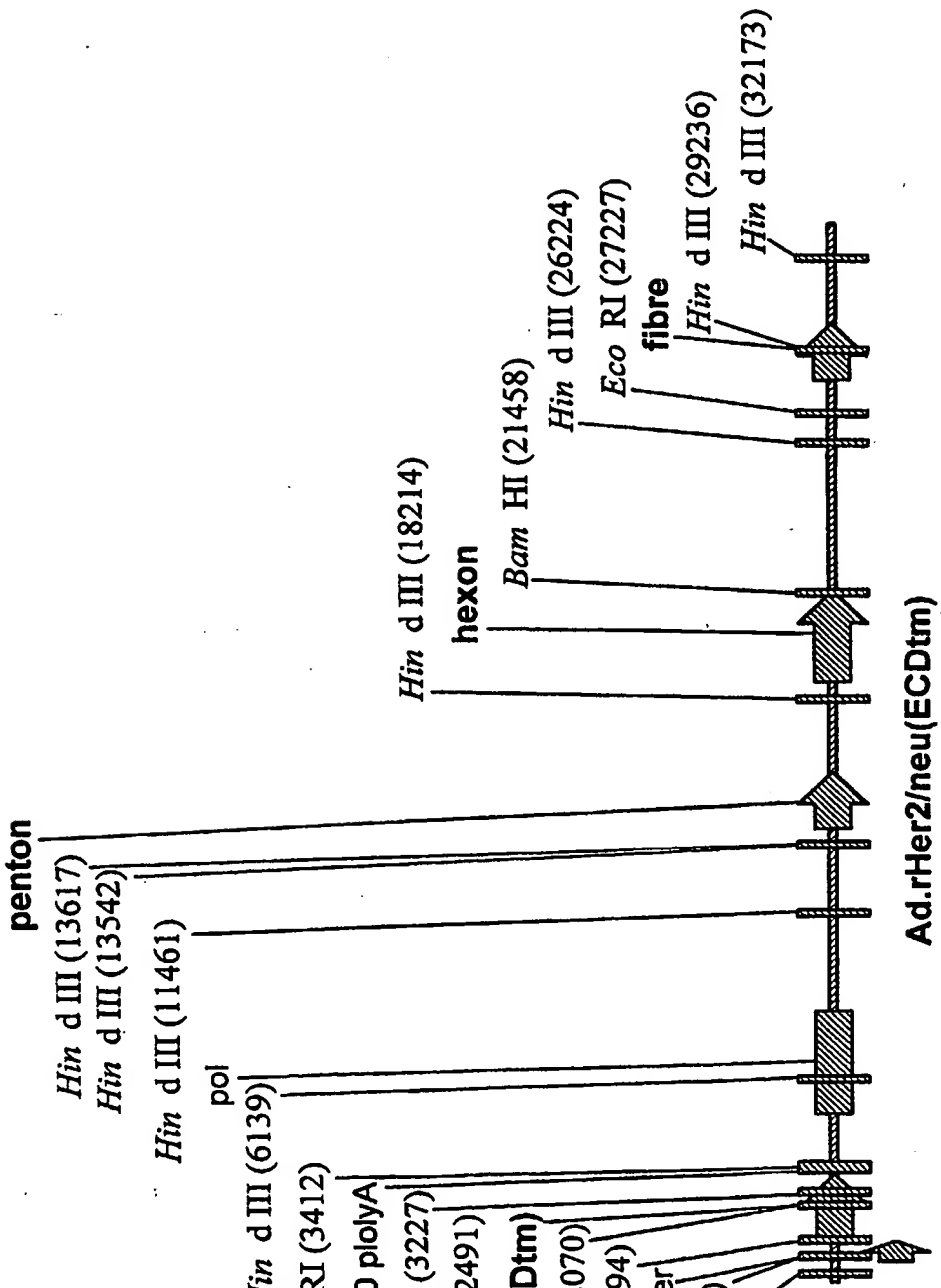
penton
Hin d III (13617)
Hin d III (13542)
Hin d III (11461)
pol
Hin d III (6139)
Eco RI (3412)
SV40 plolyA
Hin d III (3227)
Bam HI (2491)
rat Her2/neu(ECDtm)
Bam HI (2070)
Eco RI (994)
MCMV promoter
Xba I (456)
ITR
Hin d III (18214)
hexon
Bam HI (21458)
Hin d III (26224)
Eco RI (27227)
fibre
Hin d III (29236)
Hin d III (32173)
Ad.rHer2/neu(ECDtm)
33176 bp

FIG. 10

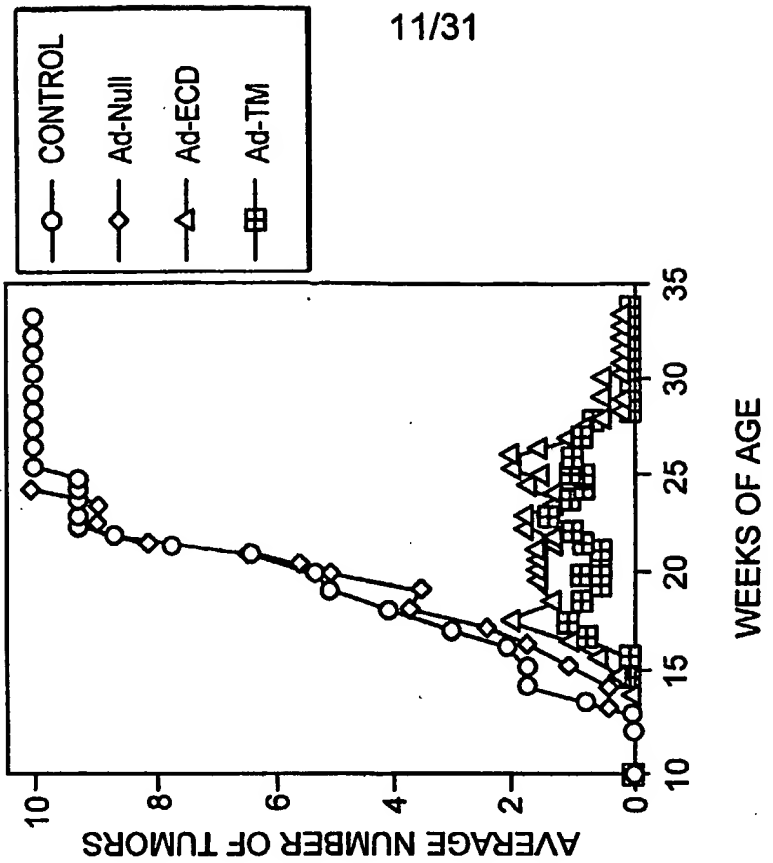

FIG. 11B

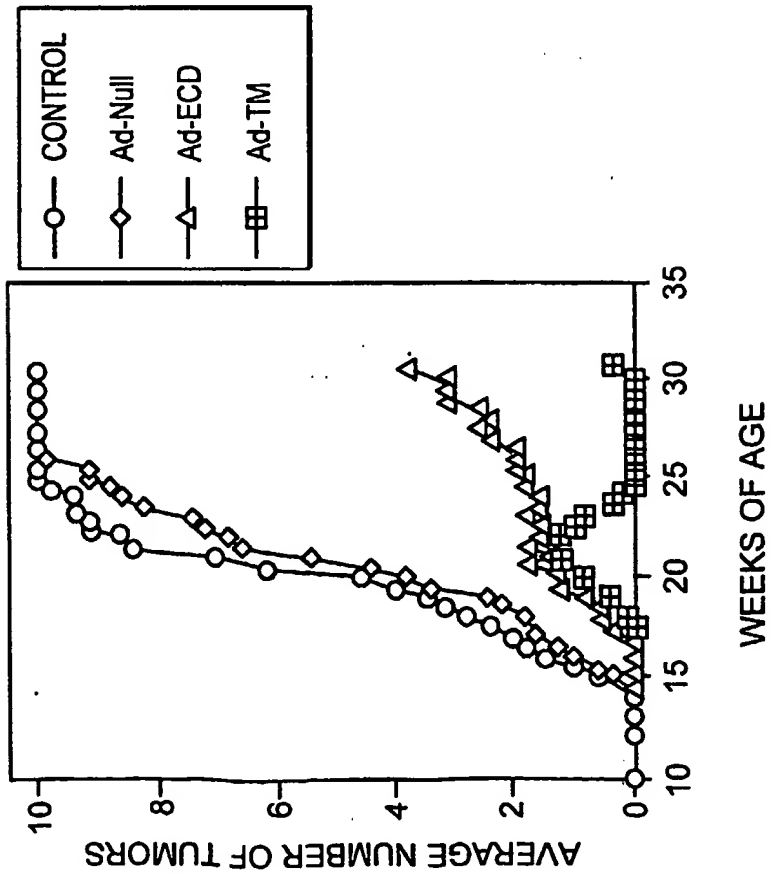

FIG. 11A

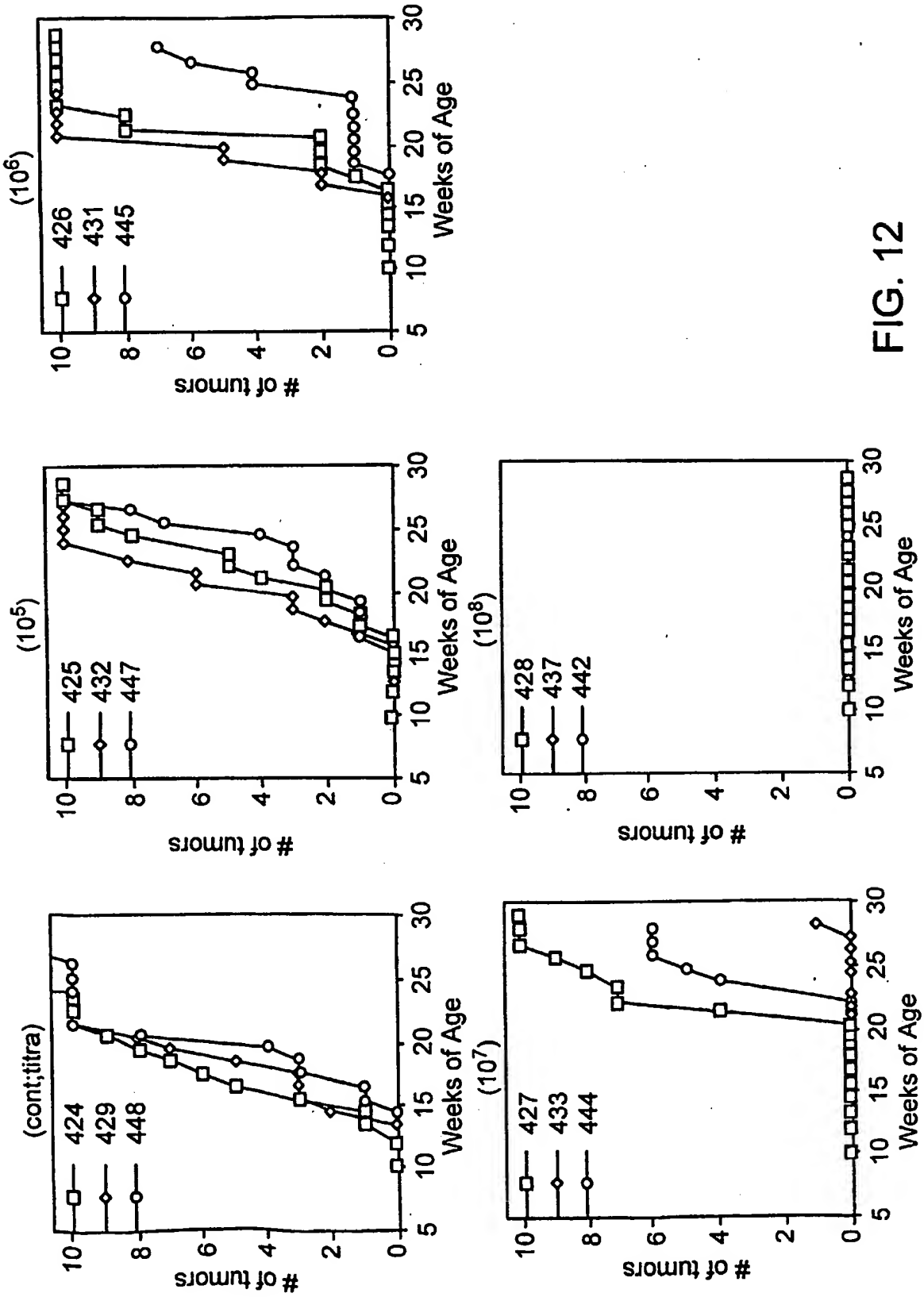
(cont;titra)
424
429
448
(10^5)
425
432
447
(10^6)
426
431
445
(10^7)
427
433
444
(10^8)
428
437
442
# of tumors
Weeks of Age
0
2
4
6
8
10
5
10
15
20
25
30

FIG. 12

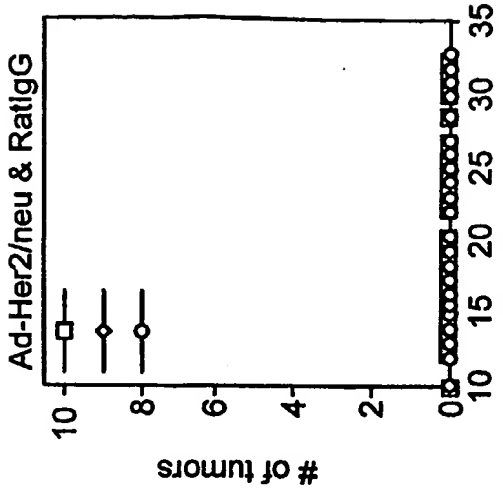

FIG. 13C

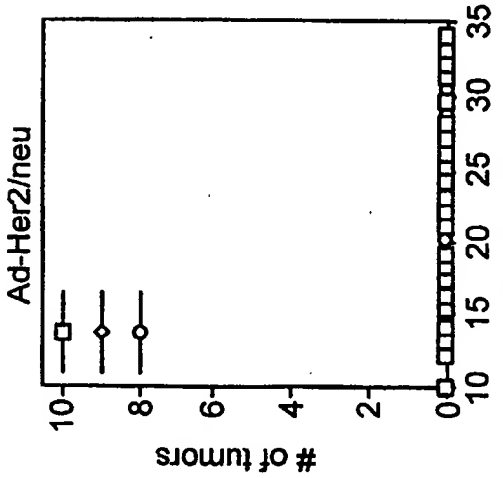

FIG. 13B

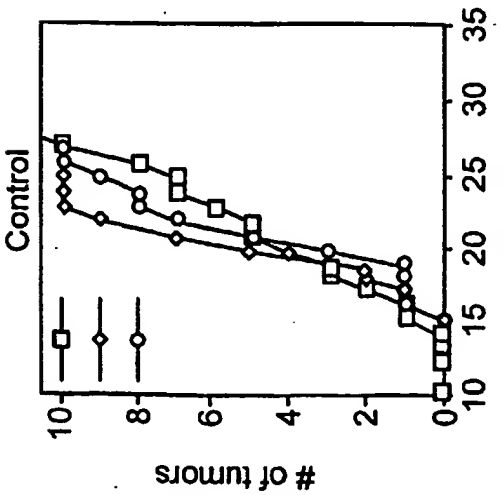

FIG. 13A

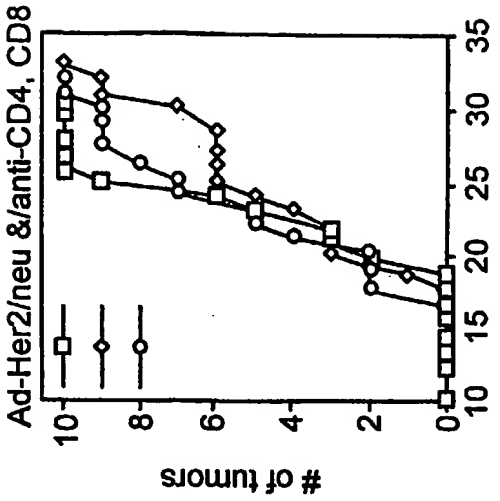

FIG. 13F

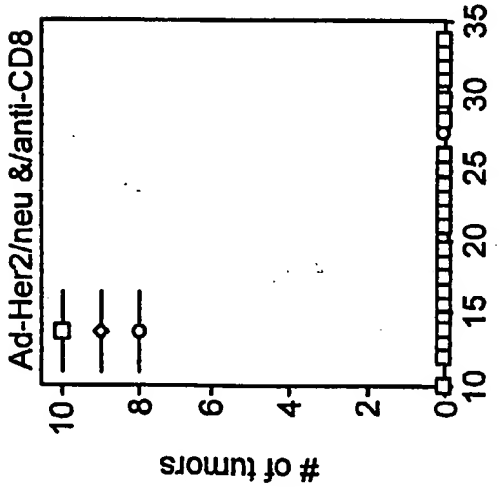

FIG. 13E

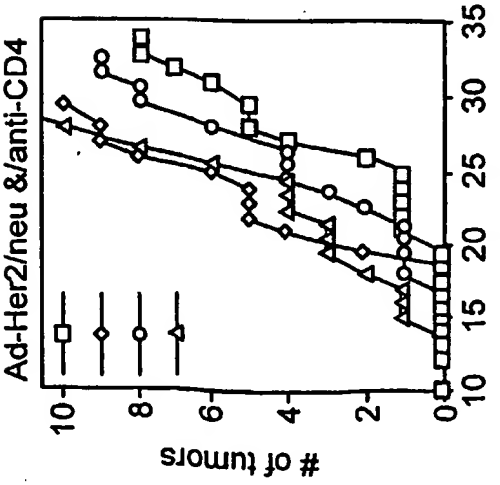

FIG. 13D

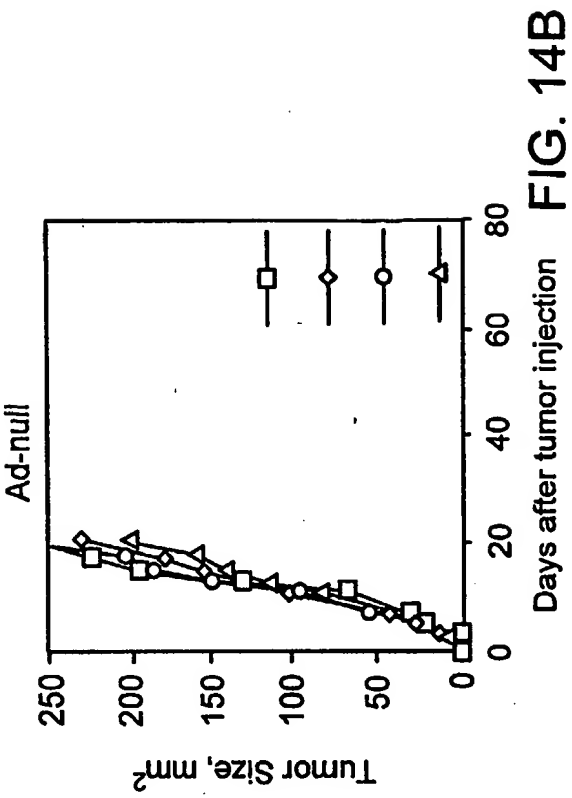

FIG. 14B

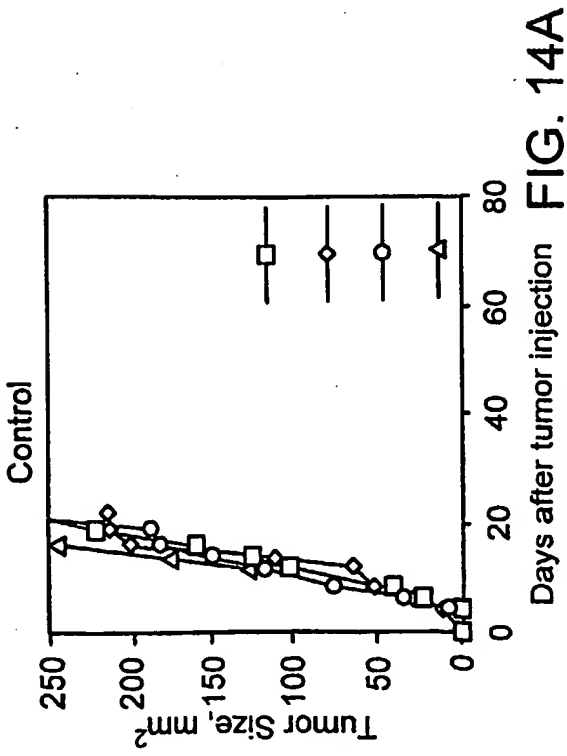

FIG. 14A

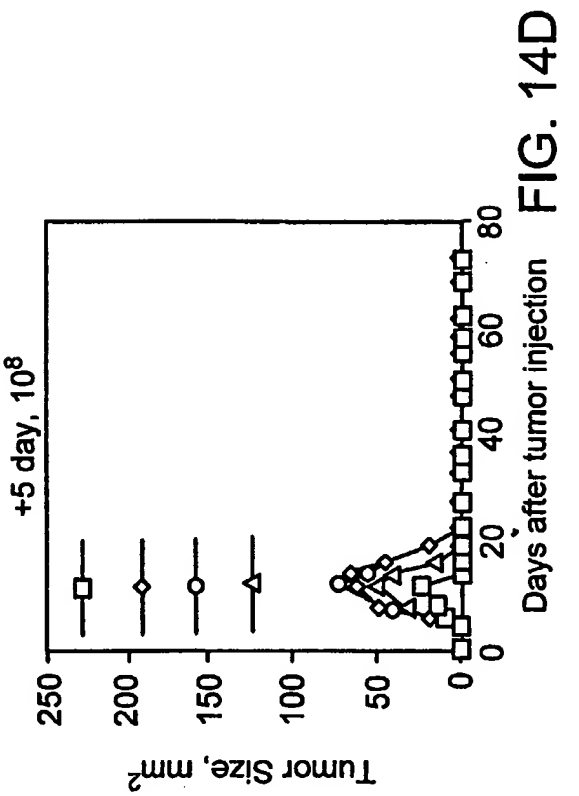

FIG. 14D

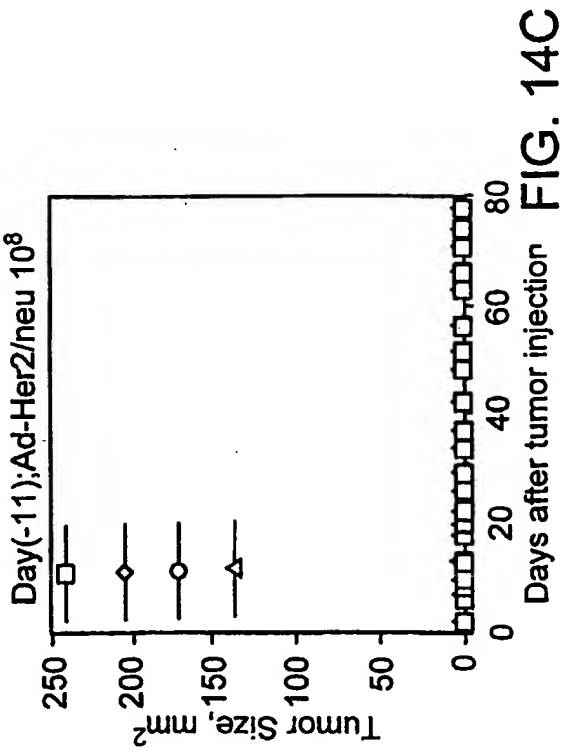

FIG. 14C

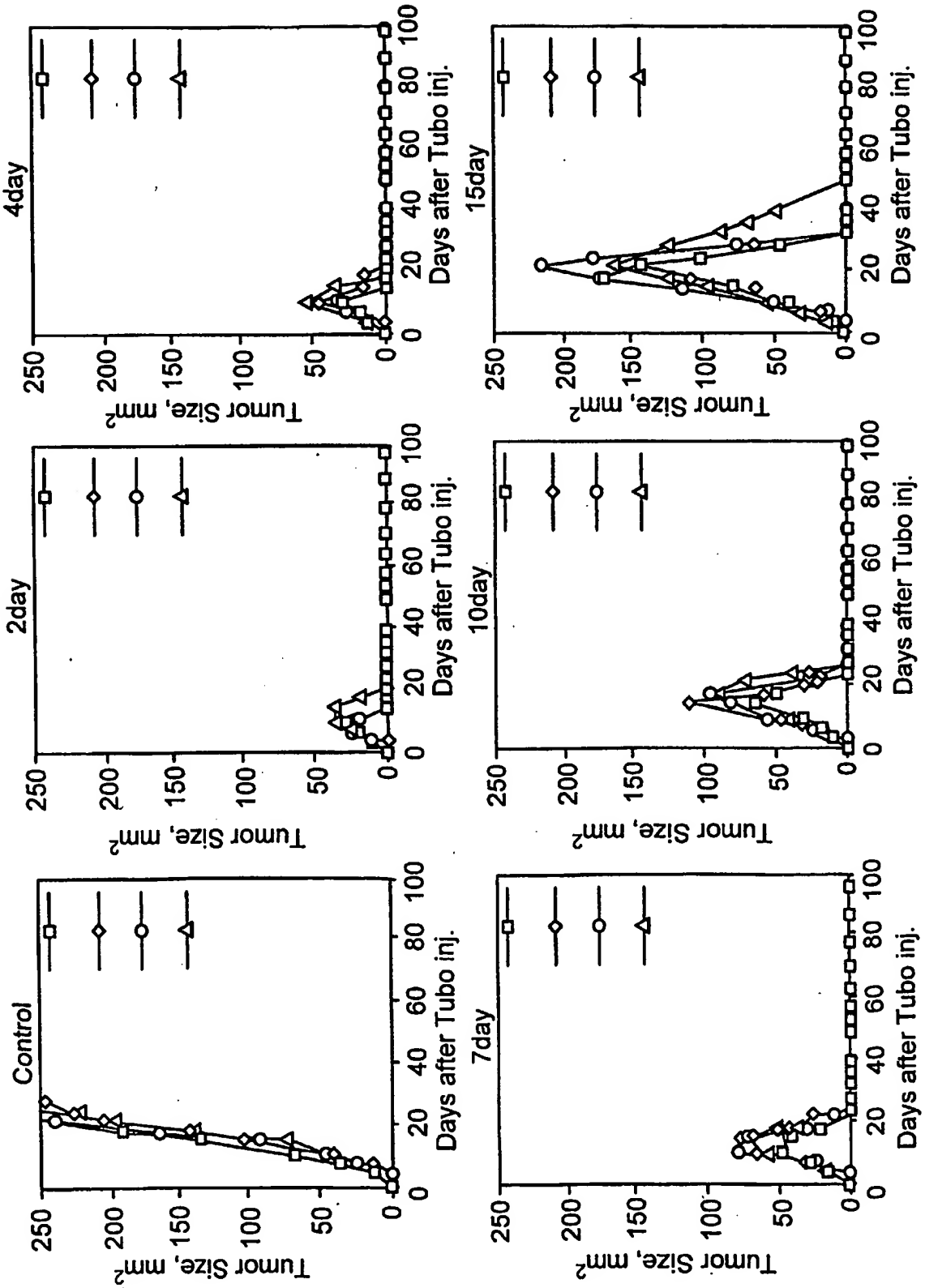
Control
2day
4day
7day
10day
15day
Tumor Size, mm2
Days after Tubo inj.
0
20
40
60
80
100
50
150
200
250

FIG. 15

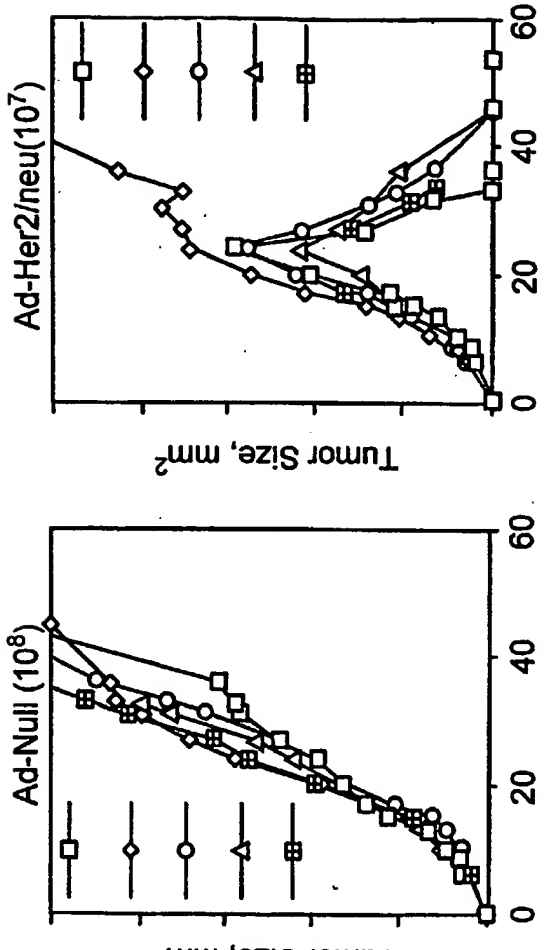

FIG. 16C

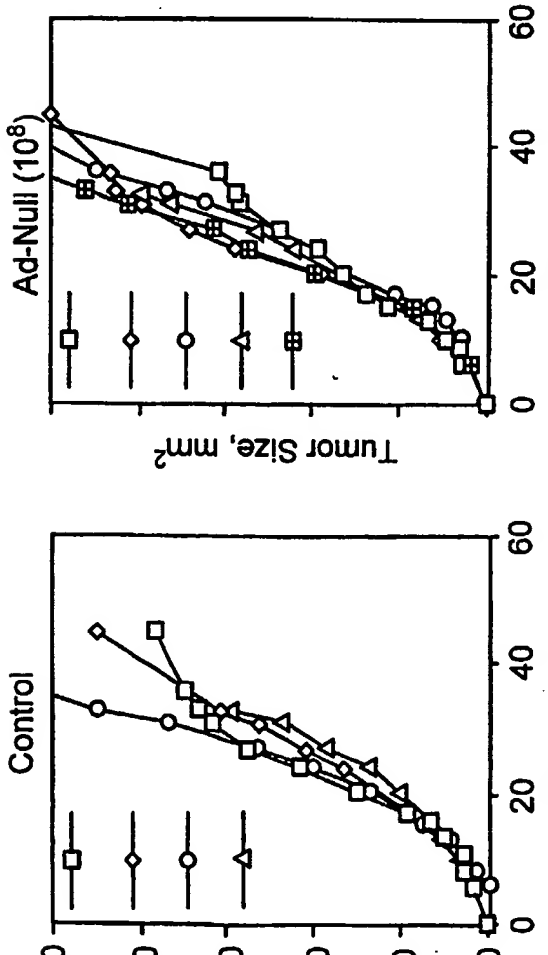

FIG. 16B

FIG. 16A

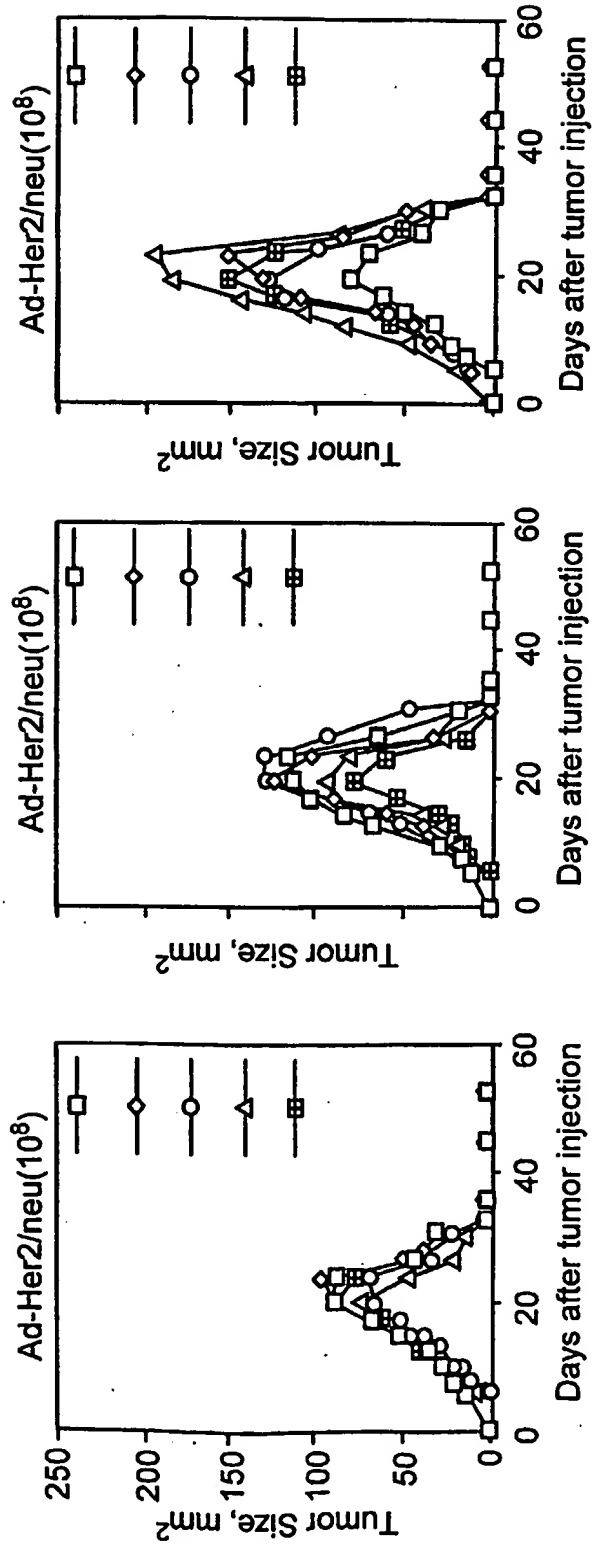

FIG. 16D

FIG. 16E

FIG. 16F

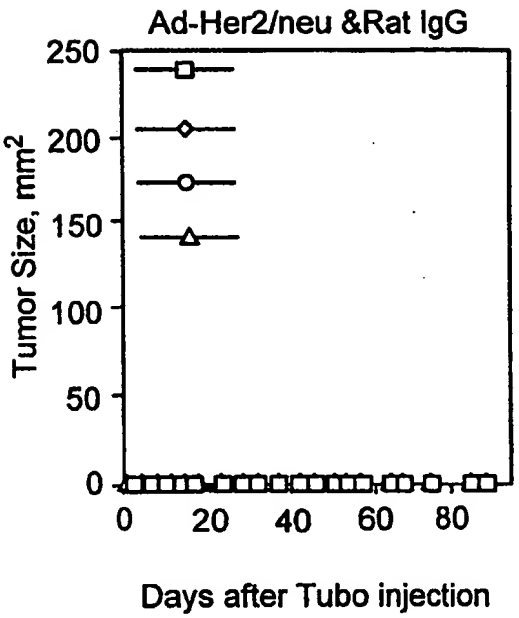

FIG. 17A

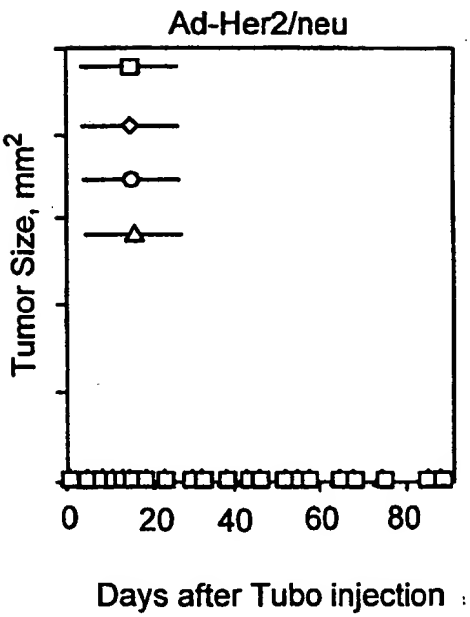

FIG. 17B

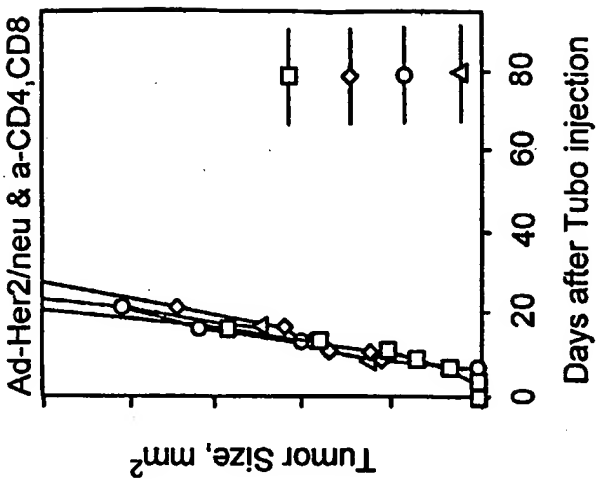

FIG. 17E

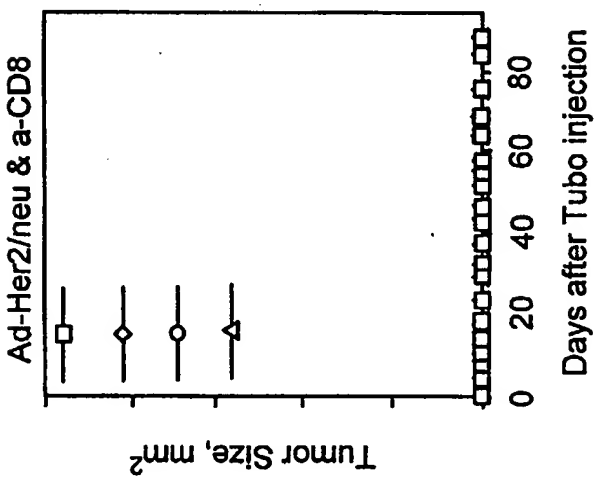

FIG. 17D

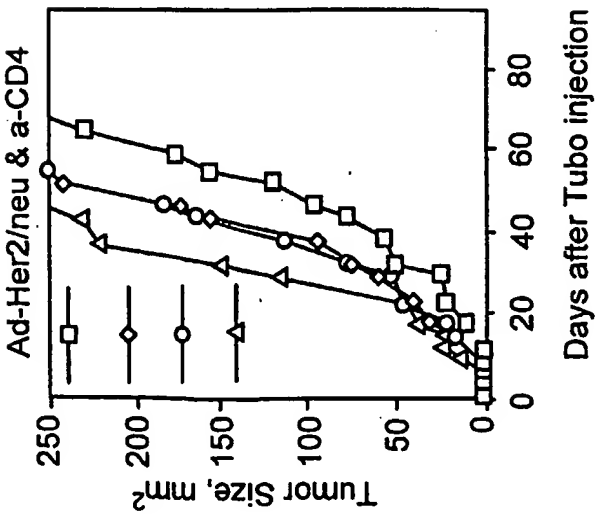

FIG. 17C

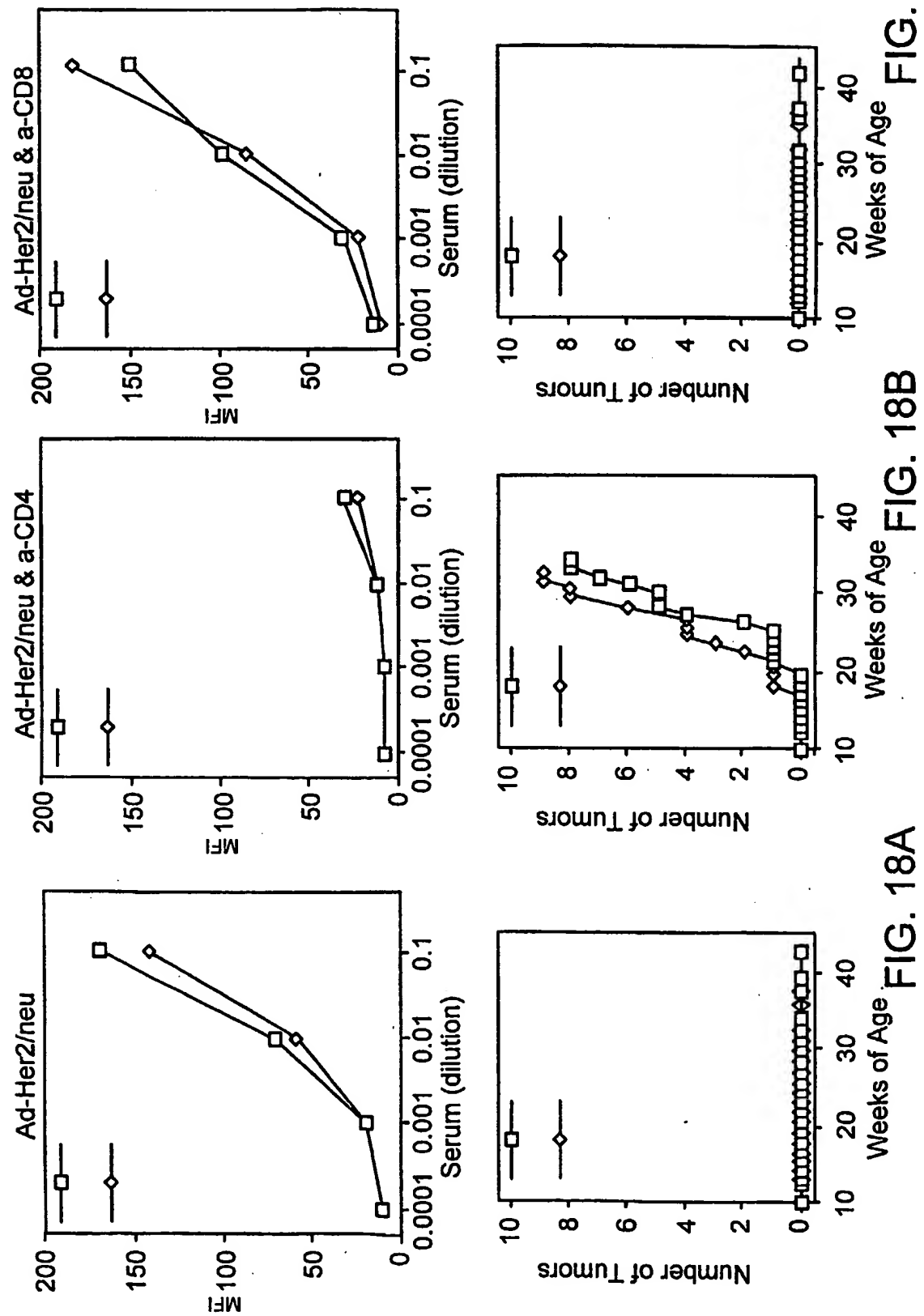

FIG. 18A

FIG. 18B

FIG. 18C

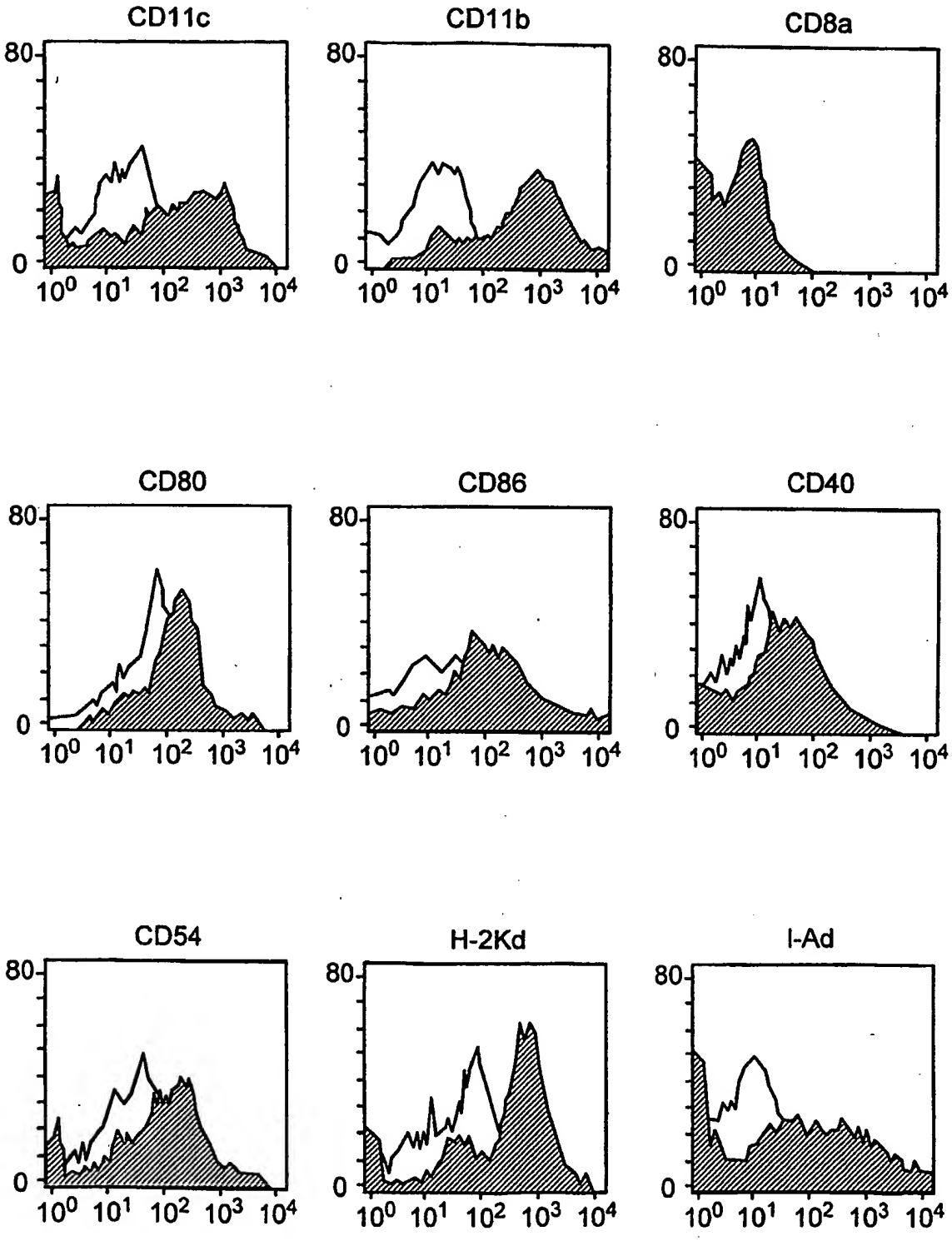
CD11c
CD11b
CD8a
CD80
CD86
CD40
CD54
H-2Kd
I-Ad
80
0
10^0 10^1 10^2 10^3 10^4

FIG. 19A

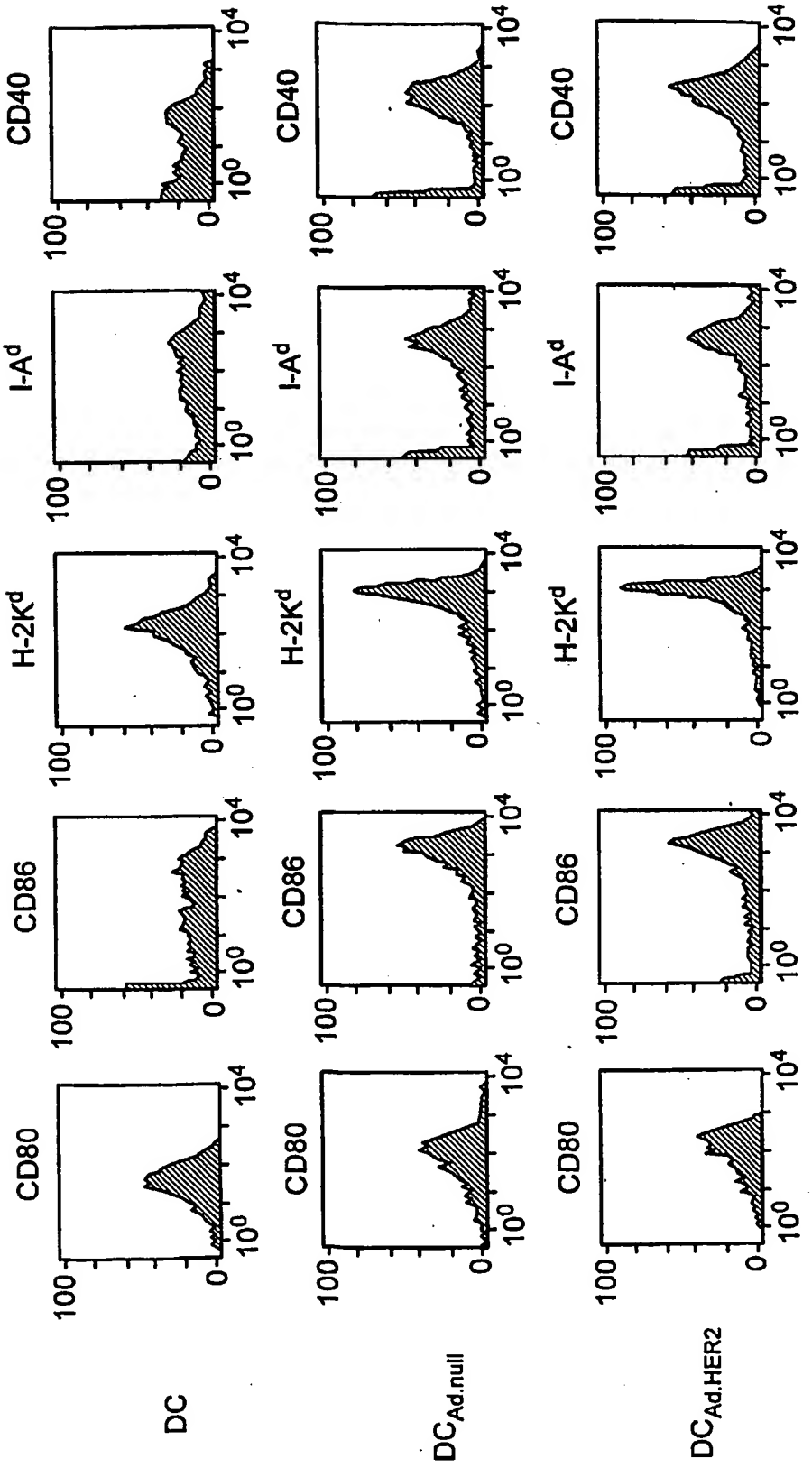

FIG. 19B

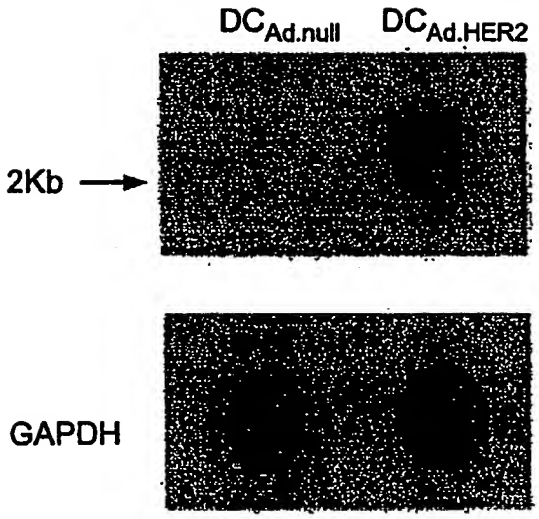

FIG. 20A

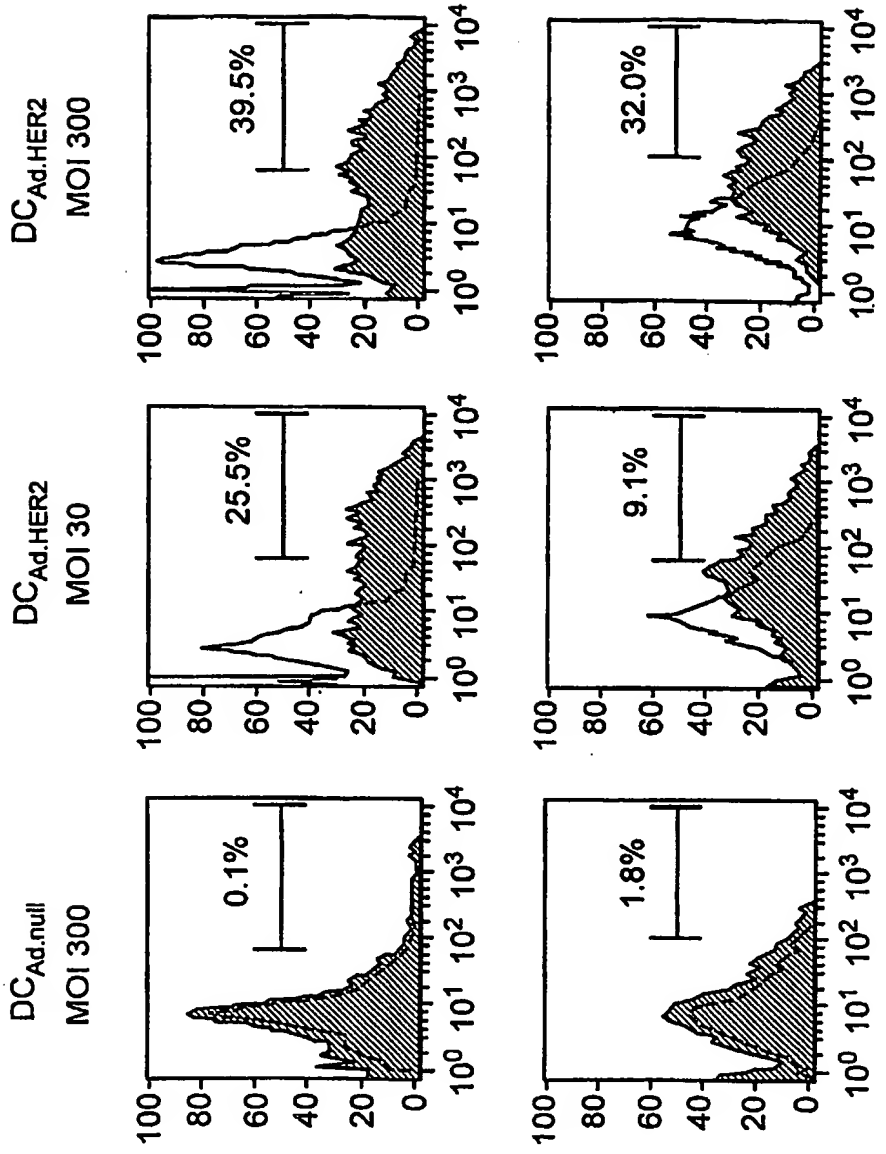
DC Ad.null MOI 300
DC Ad.HER2 MOI 30
DC Ad.HER2 MOI 300
PERMEABILIZED
NON-PERMEABILIZED
0.1%
25.5%
39.5%
1.8%
9.1%
32.0%

FIG. 20B

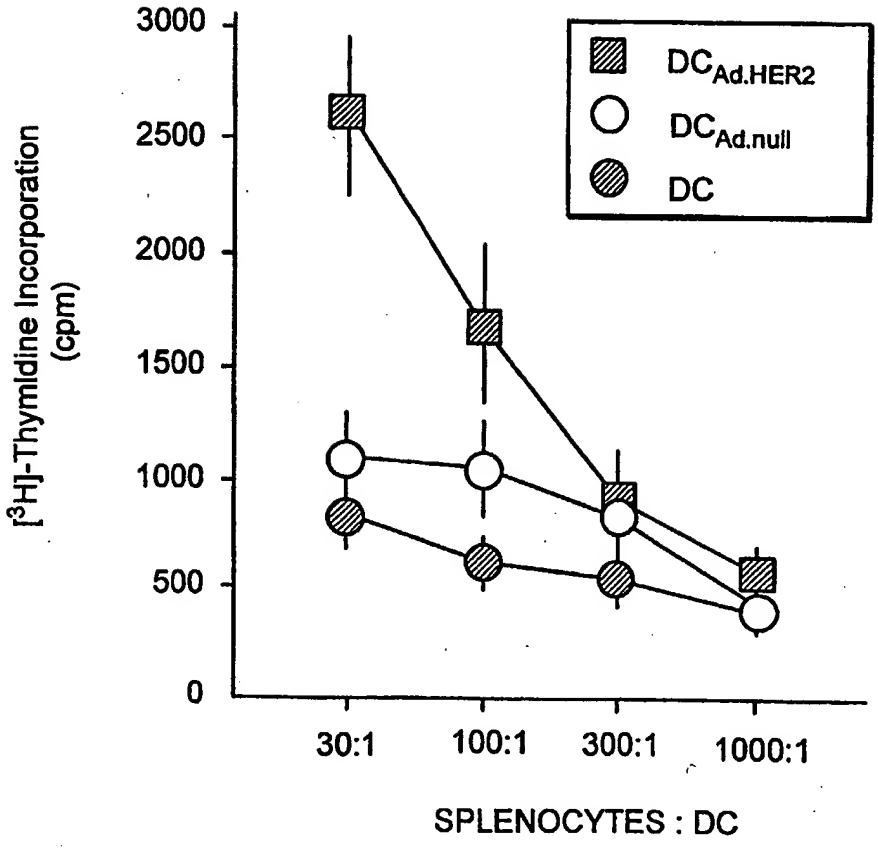
3000
2500
2000
1500
1000
500
0
[3H]-Thymidine Incorporation (cpm)
DCAd.HER2
DCAd.null
DC
30:1
100:1
300:1
1000:1
SPLENOCYTES : DC

FIG. 21

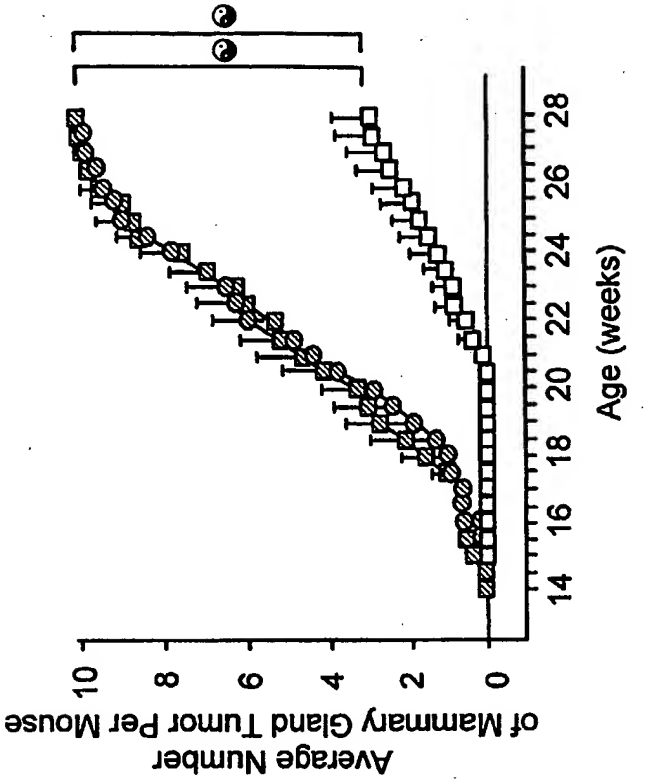

FIG. 22B

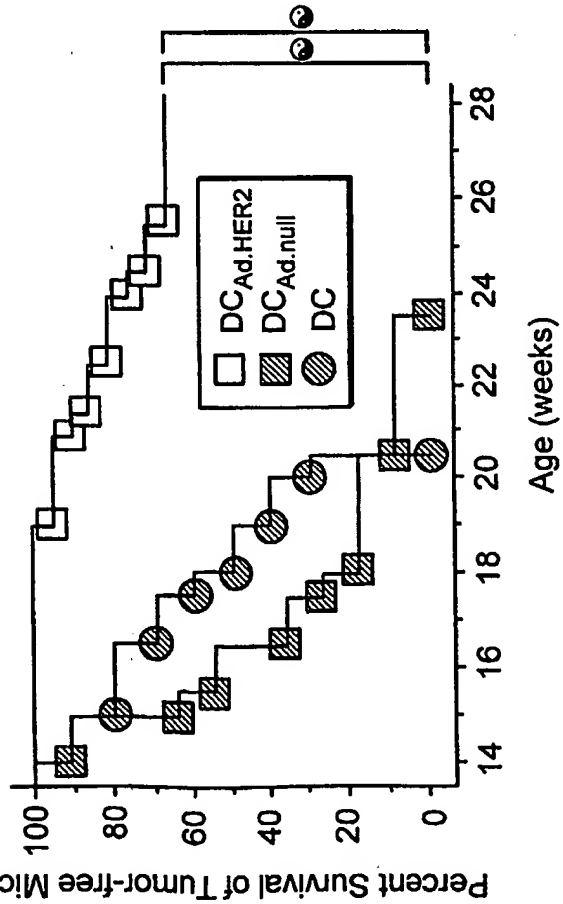

FIG. 22A

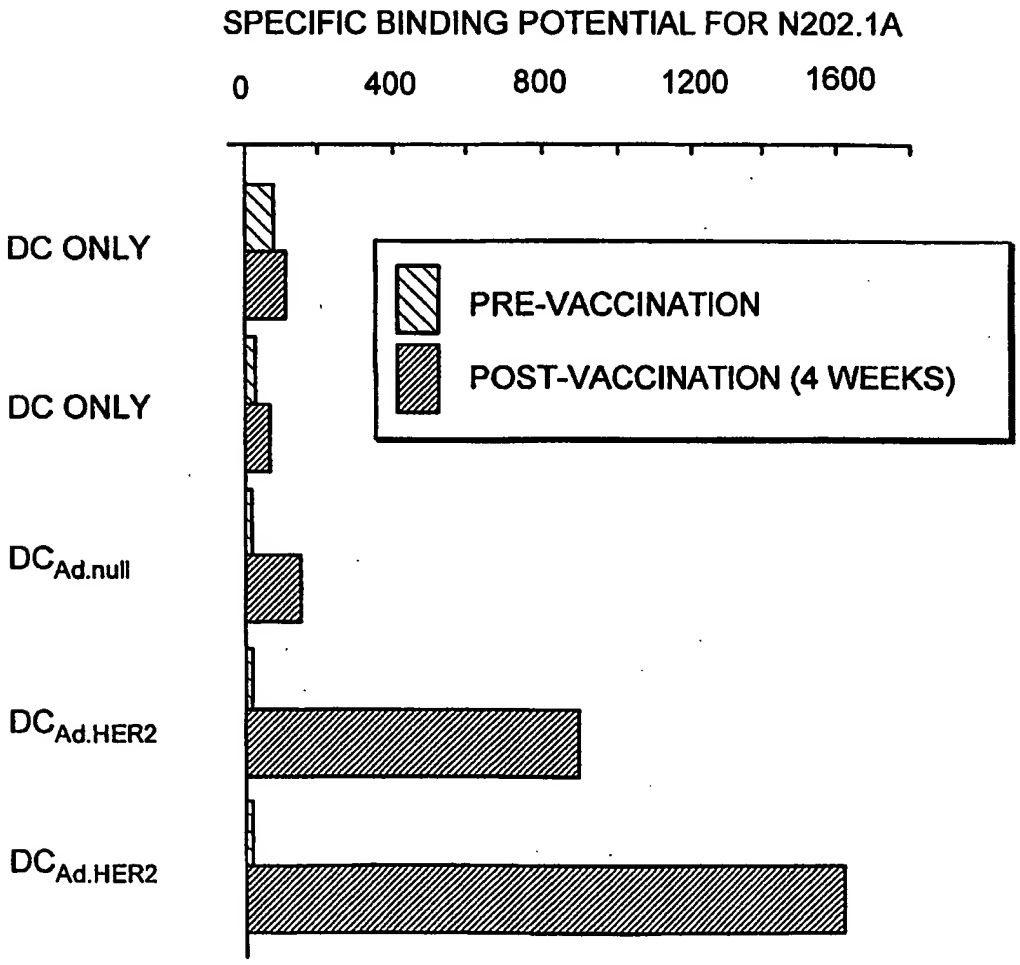
SPECIFIC BINDING POTENTIAL FOR N202.1A
0
400
800
1200
1600
DC ONLY
DC ONLY
DC Ad.null
DC Ad.HER2
DC Ad.HER2
PRE-VACCINATION
POST-VACCINATION (4 WEEKS)

FIG. 23A

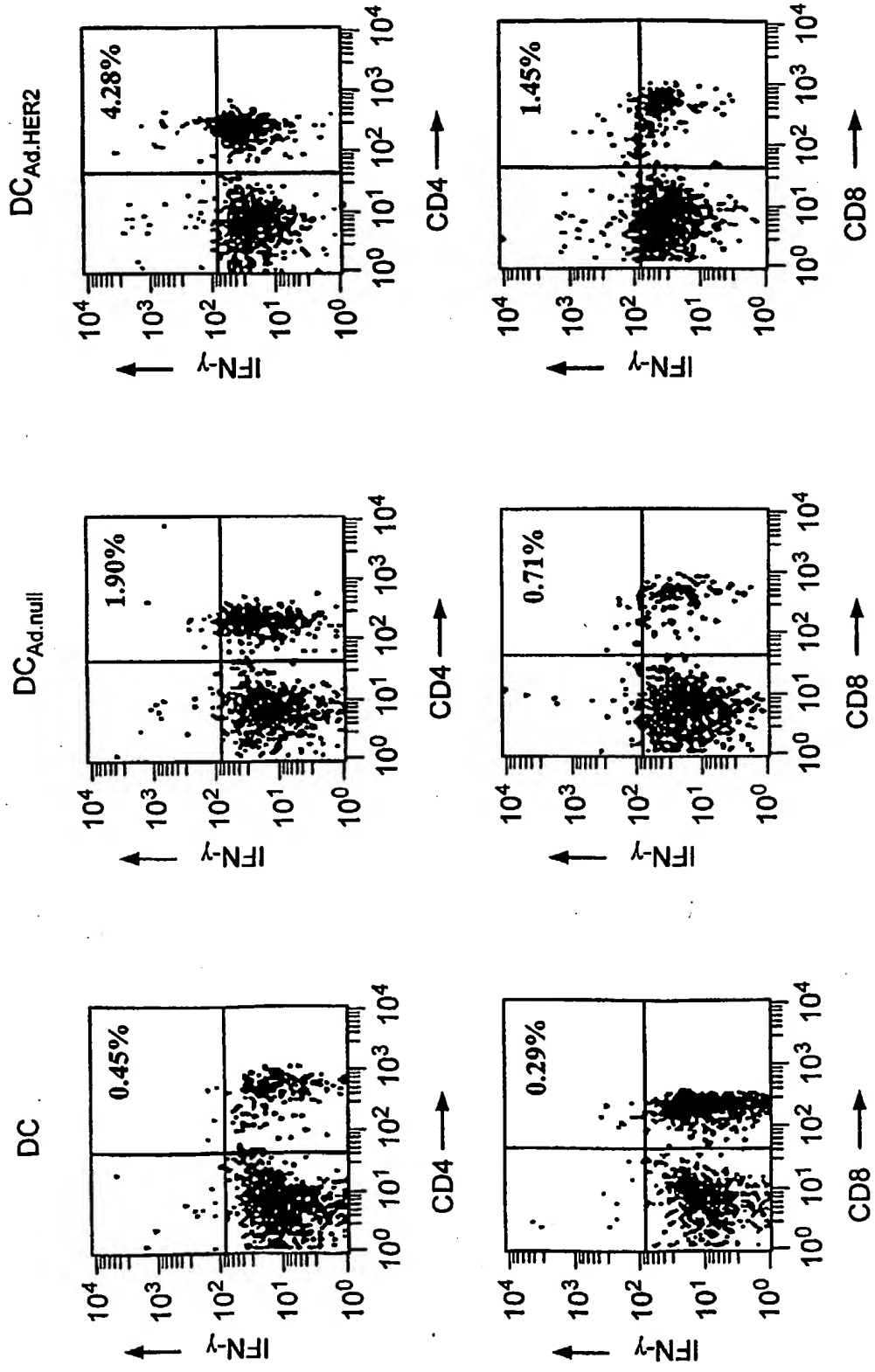
DC
DC$_{Ad.null}$
DC$_{Ad.HER2}$
0.45%
1.90%
4.28%
0.29%
0.71%
1.45%
IFN-γ
CD4
CD8
10^0 10^1 10^2 10^3 10^4

FIG. 23B

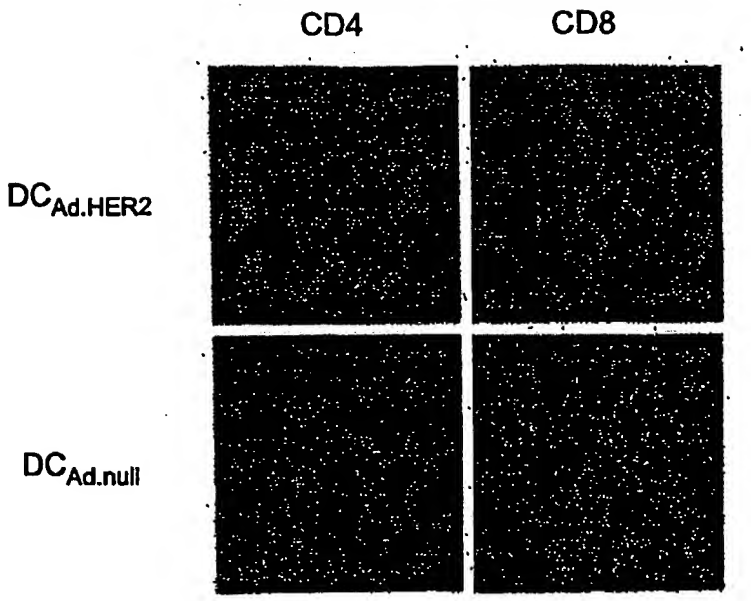
CD4
CD8
DC Ad.HER2
DC Ad.null

FIG. 24

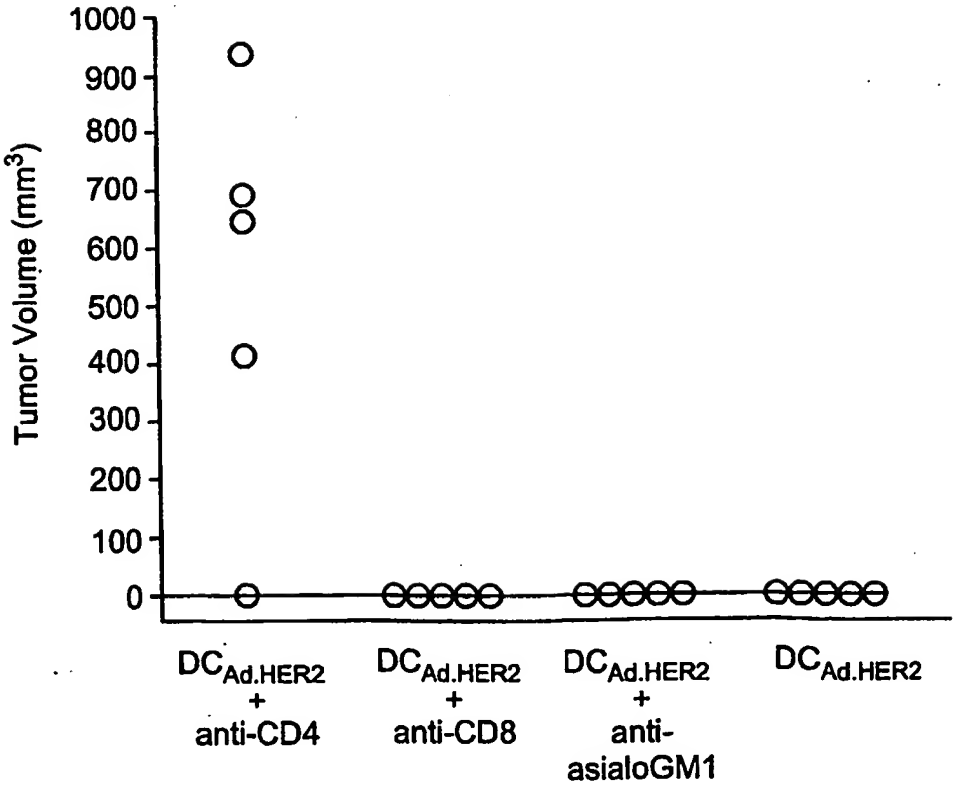
Tumor Volume (mm3)
1000
900
800
700
600
500
400
300
200
100
0
DC Ad.HER2 + anti-CD4
DC Ad.HER2 + anti-CD8
DC Ad.HER2 + anti-asialoGM1
DC Ad.HER2

FIG. 25

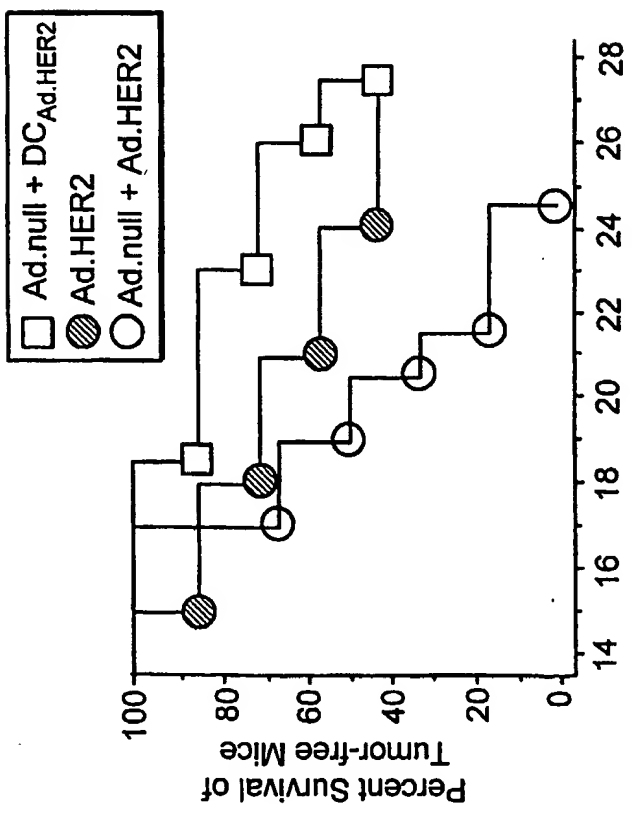

FIG. 26A

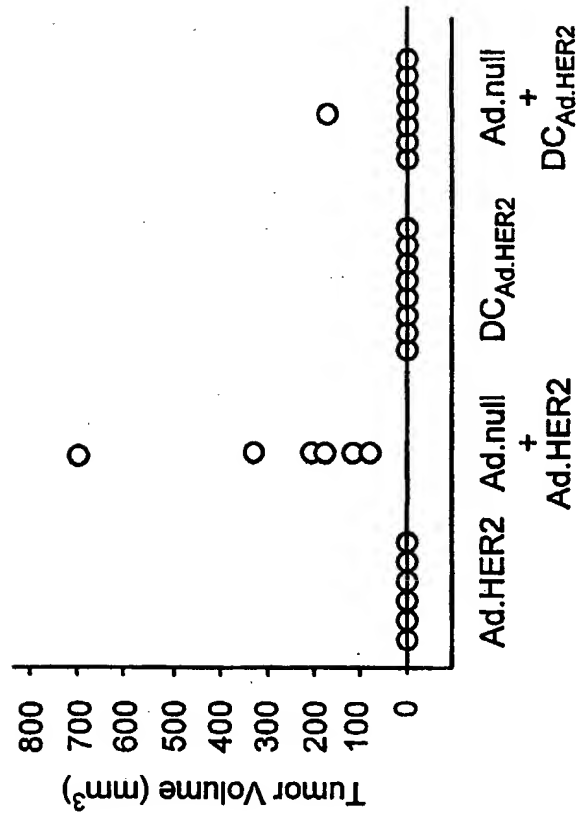

FIG. 26B

SEQUENCE LISTING

<110> THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN

<120> METHODS FOR PROPHYLAXIS AND TREATMENT OF HER-2/neu TUMORS

<130> 58342-PCT(47992)

<140> PCT/US03/34362
<141> 2003-10-29

<150> 60/422,395
<151> 2002-10-30

<160> 2

<170> PatentIn Ver. 3.2

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<211> 2079
<212> DNA
<213> Rattus sp.

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cccggaatcg cgggcaccca agtgtgtacc ggcacagaca tgaagttgcg gctccctgcc 120
agtcctgaga cccacctgga catgctccgc cacctgtacc agggctgtca ggtagtgcag 180
ggcaacttgg agcttaccta cgtgcctgcc aatgccagcc tctcattcct gcaggacatc 240
caggaagttc agggttacat gctcatcgct cacaaccagg tgaagcgcgt cccactgcaa 300
aggctgcgca tcgtgagagg gacccagctc tttgaggaca agtatgccct ggctgtgcta 360
gacaaccgag atcctcagga caatgtcgcc gcctccaccc caggcagaac cccagagggg 420
ctgcgggagc tgcagcttcg aagtctcaca gagatcctga agggaggagt tttgatccgt 480
gggaaccctc agctctgcta ccaggacatg gttttgtgga aggacgtctt ccgcaagaat 540
aaccaactgg ctcctgtcga tatagacacc aatcgttccc gggcctgtcc accttgtgcc 600
cccgcctgca aagacaatca ctgttggggt gagagtccgg aagactgtca gatcttgact 660
ggcaccatct gtaccagtgg ttgtgcccgg tgcaagggcc ggctgcccac tgactgctgc 720
catgagcagt gtgccgcagg ctgcacgggc cccaagcatt ctgactgcct ggcctgcctc 780
cacttcaatc atagtggtat ctgtgagctg cactgcccag ccctcgtcac ctacaacaca 840
gacacctttg agtccatgca caaccctgag ggtcgctaca cctttggtgc cagctgcgtg 900
accacctgcc cctacaacta cctgtctacg gaagtgggat cctgcactct ggtgtgtccc 960
ccgaataacc aagaggtcac agctgaggac ggaacacagc gttgtgagaa atgcagcaag 1020
ccctgtgctc gagtgtgcta tggtctgggc atggagcacc ttcgaggggc gagggccatc 1080
accagtgaca atgtccagga gtttgatggc tgcaagaaga tctttgggag cctggcattt 1140
ttgccggaga gctttgatgg ggacccctcc tccggcattg ctccgctgag gcctgagcag 1200
ctccaagtgt tcgaaaccct ggaggagatc acaggttacc tgtacatctc agcatggcca 1260
gacagtctcc gtgacctcag tgtcttccag aaccttcgaa tcattcgggg acggattctc 1320
cacgatggcg cgtactcatt gacactgcaa ggcctgggga tccactcgct ggggctgcgc 1380
tcactgcggg agctgggcag tggattggct ctgattcacc gcaacgccca tctctgcttt 1440
gtacacactg taccttggga ccagctcttc cggaacccac atcaggccct gctccacagt 1500
gggaaccggc cggaagagga cttgtgcgtc tcgagcggct tggtctgtaa ctcactgtgt 1560
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